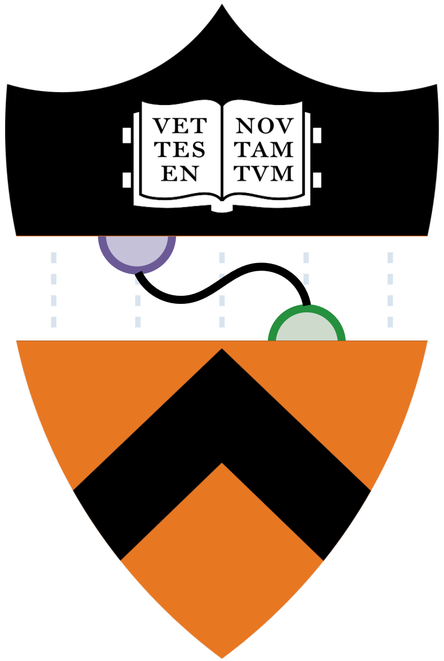
Induced Proximity

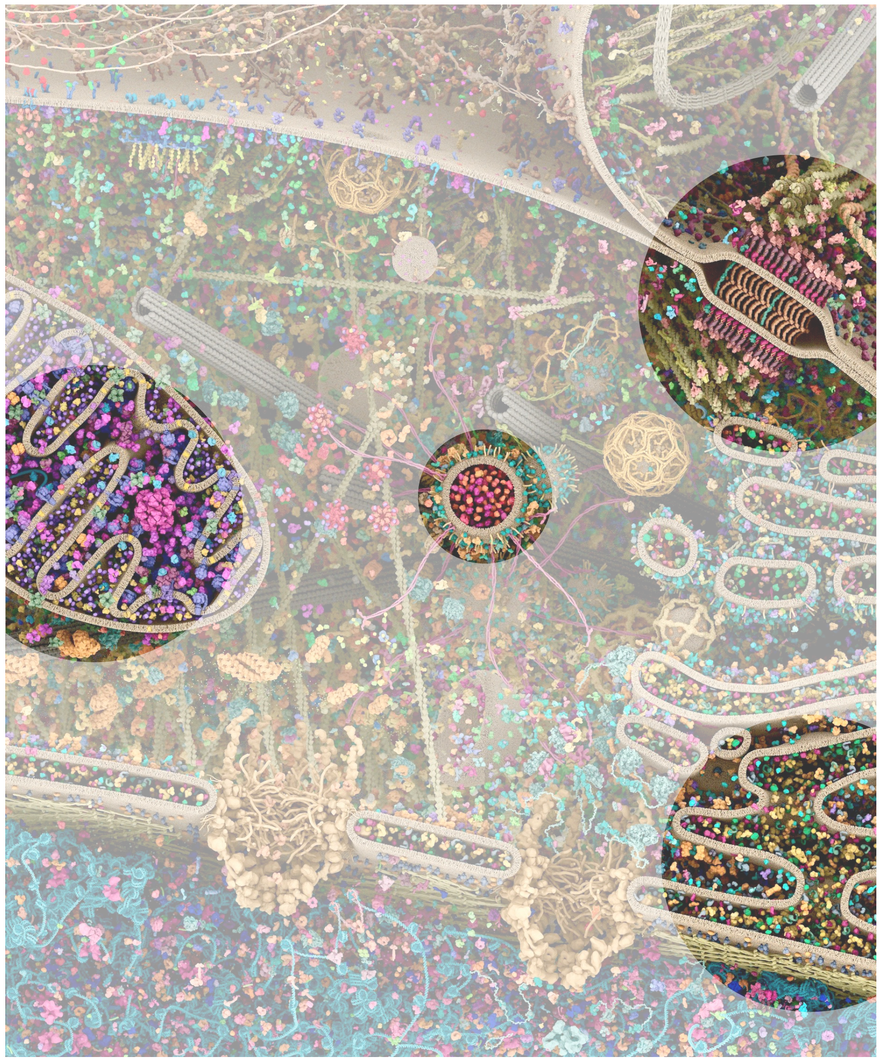


Philip Raftopoulos

Group meeting: February 7th, 2025

Reactions in biology are incredibly impacted by distance

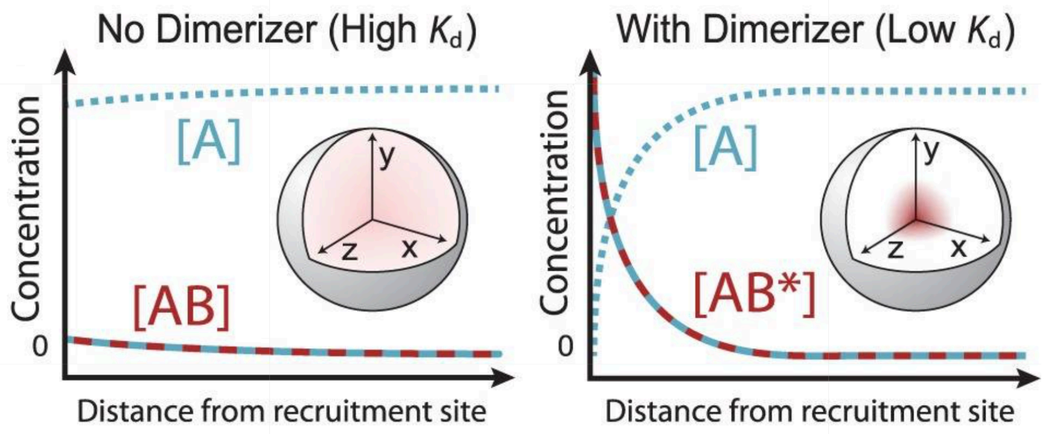
Reactions are regulated by distinct sub-cellular localization



Stanton, B. Z.; Chory, E. J.; Crabtree, G. R. *Science* **2018**, *359* (6380).

Reactions in biology are incredibly impacted by distance

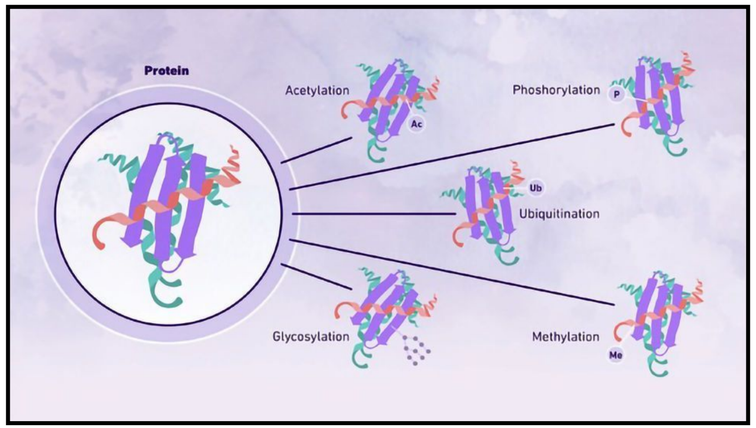
Cells leverage a specific principle of diffusion in order to regulate reactions



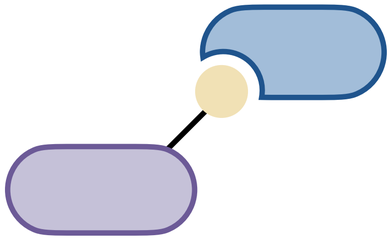
Increasing affinity, decreases reaction radius!!

Stanton, B. Z.; Chory, E. J.; Crabtree, G. R. *Science* **2018**, *359* (6380).

Reactions in biology are incredibly impacted by distance



Post translational modifications

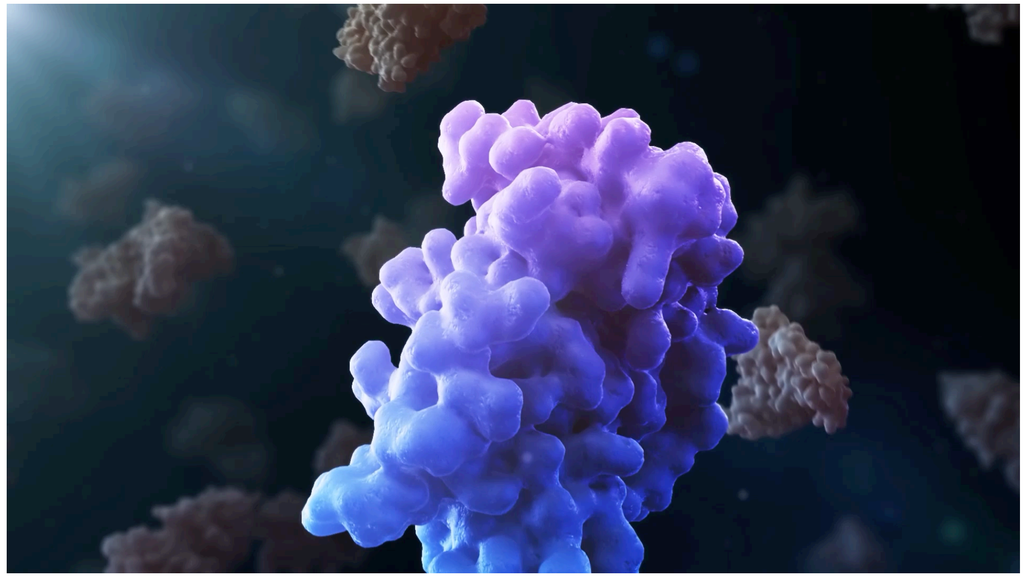


Proximity induced upon PTMs

Spatially and temporally regulated sites of reaction

Stanton, B. Z.; Chory, E. J.; Crabtree, G. R. *Science* **2018**, *359* (6380).

As chemists, how can we leverage induced proximity?

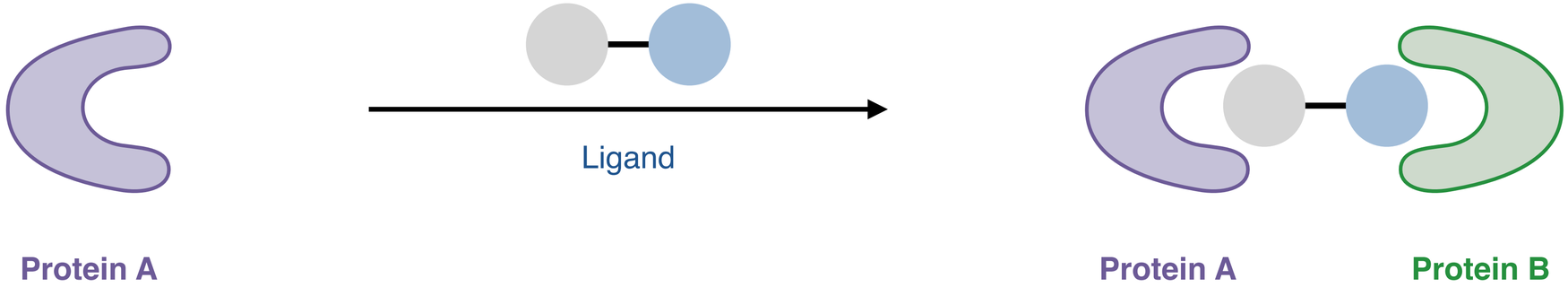


- **Study cell signaling**
- **Design new modes of therapy**

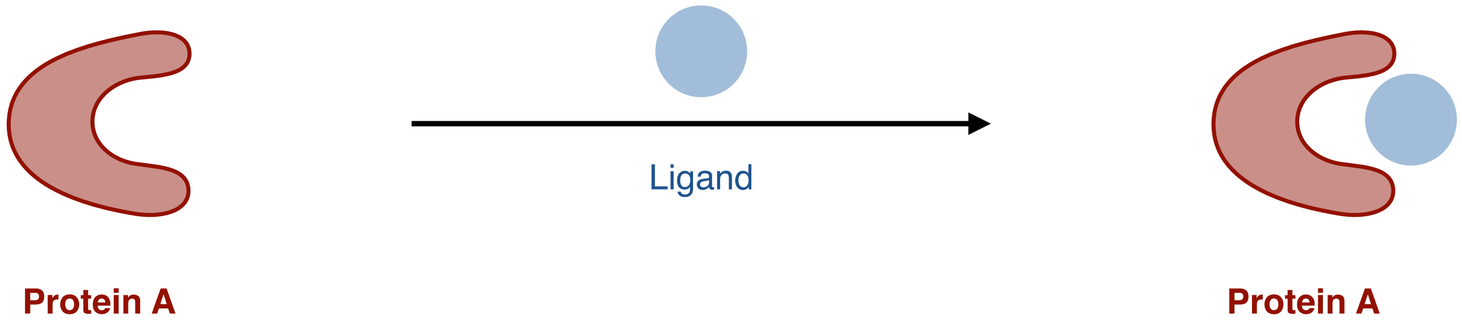
Design of ligands that artificially induce proximity between proteins

What is induced proximity?

Chemical inducers of proximity

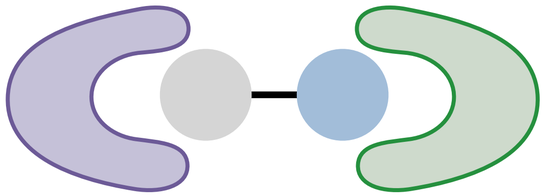


Traditional small molecules

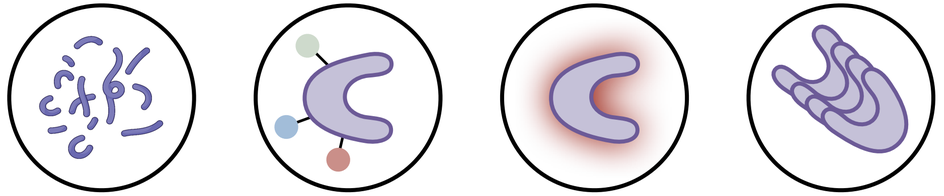


What is induced proximity?

Chemical inducers of proximity



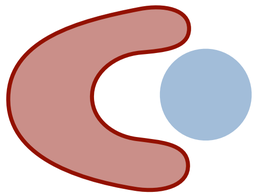
Protein A **Protein B**



Degradation *PTMs* *Inhibition* *Polymerization*

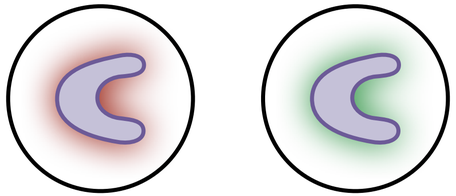
AND MORE

Traditional small molecules



Protein A

MAYBE

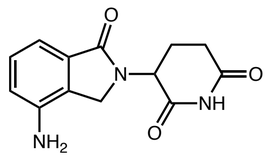


Inhibition *Agonism*

What is induced proximity?

Event-driven pharmacology

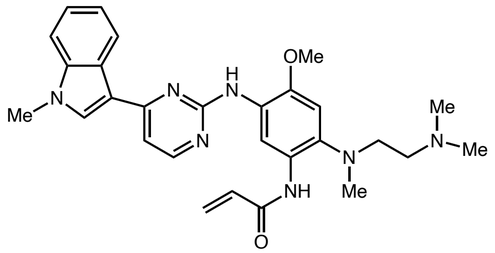
- One molecule, multiple events
- Does not require an active site



Lenalidomide

Traditional pharmacology

- Driven by **stoichiometry**
- Requires a “ligandable” pocket



Osimertinib

Békés, M.; Langley, D. R.; Crews, C. M. *Nat Rev Drug Discov* **2022**, *21* (3), 181–200.

Outline of talk:

- **History of induced proximity**
- **Modern design of chemical inducers of proximity**
- **Clinical outlook of induced proximity**

History of induced proximity

Millions of years ago

Hormones in plants

Auxin and photo-tropism

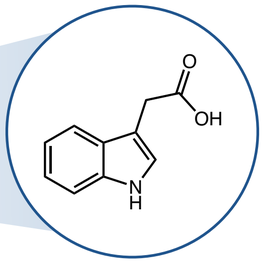
Békés, M.; Langley, D. R.; Crews, C. M. *Nat Rev Drug Discov* **2022**, *21* (3), 181–200.

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

Nature utilizes induced proximity in ancient processes

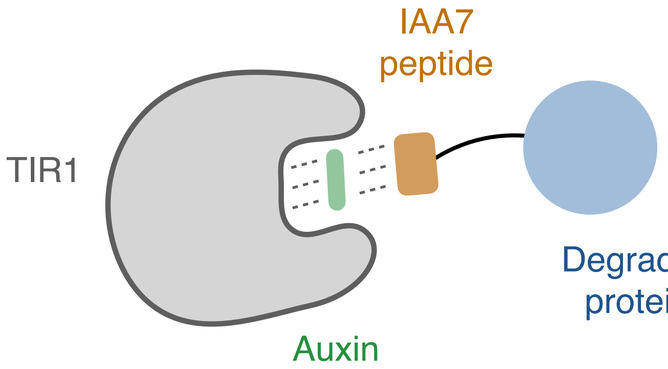
Photo-tropism

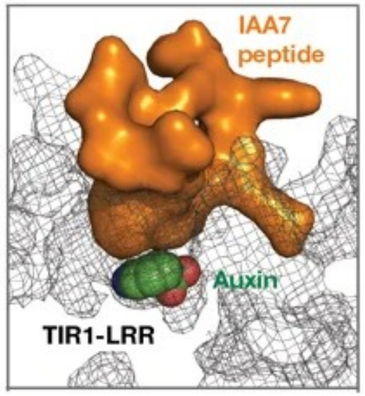
The act of moving towards light



Auxin

Concentrated in regions closest to the sun



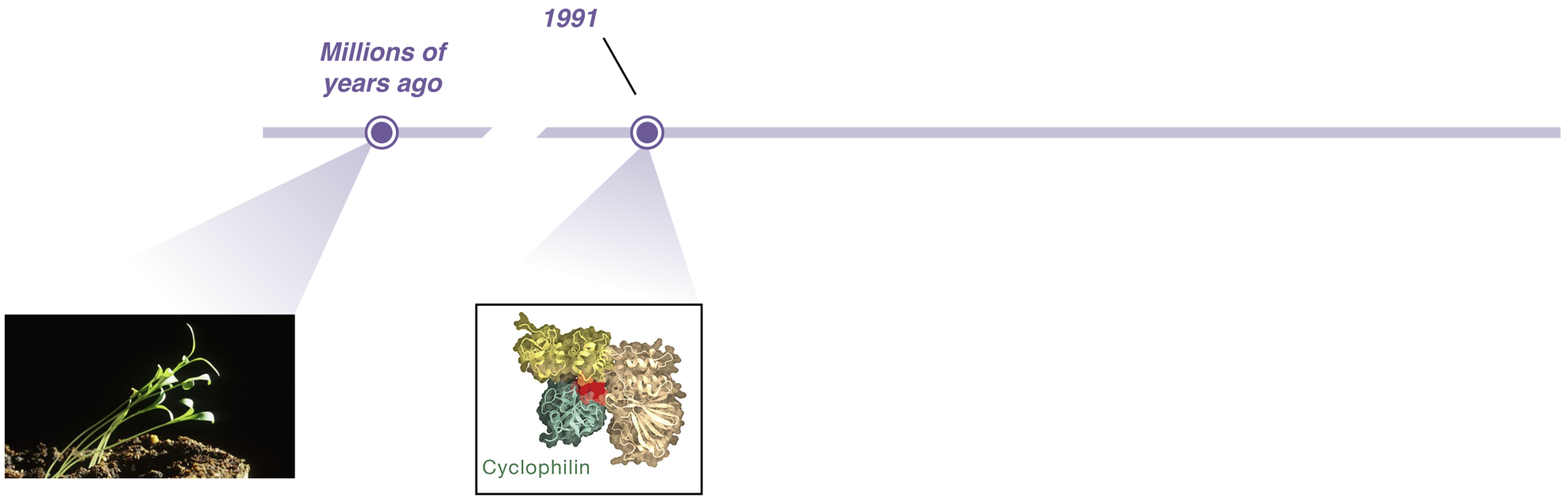


*Auxin enhances the interaction between **TIR1** and **IAA7** peptides*

Transcriptional changes

Tan, X. et. al. *Nature* **2007**, *446*, 640–645.

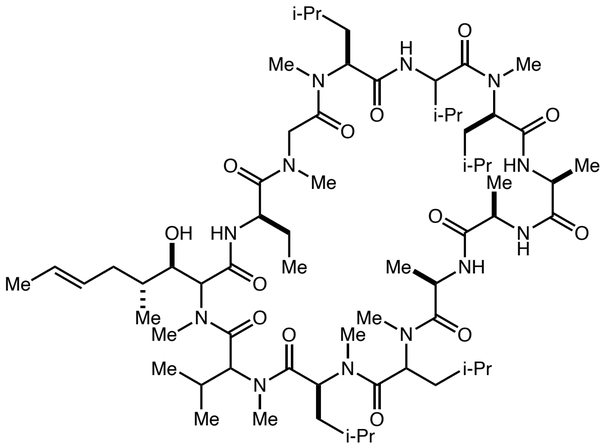
History of induced proximity



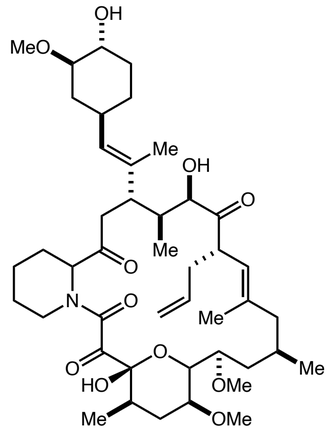
Békés, M.; Langley, D. R.; Crews, C. M. *Nat Rev Drug Discov* **2022**, *21* (3), 181–200.

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

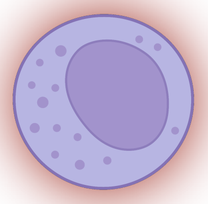
Cyclosporine and FK-506



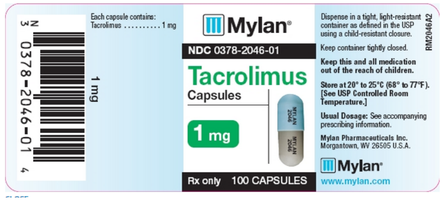
Cyclosporin A



FK-506



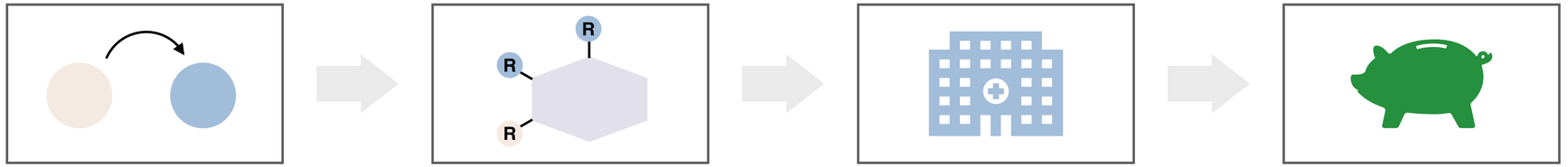
Immune inactivation



Utilized for organ transplantation

BUT possesses key side effects

Cyclosporine and FK-506



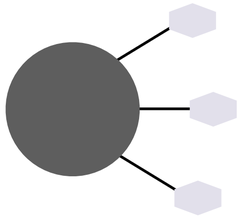
Understand mechanism → *Derivatize* → *Better safety / efficacy* → *$$$$*

Stuart Schreiber
Collaboration with Vertex

→ **How do cyclosporine A and FK-506 work?**

Initial mechanistic evidence

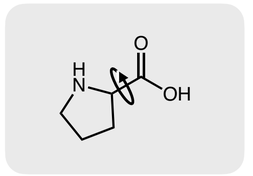
Affinity purification



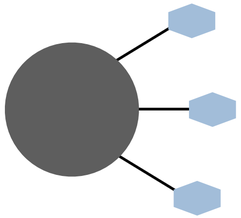
Cyclosporine beads

Cell extract

Cyclophilin



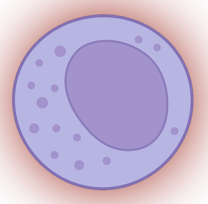
Proline isomerases



FK-506 beads

Cell extract

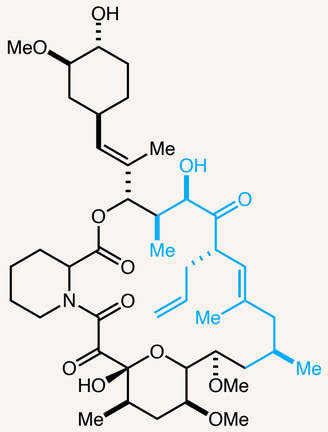
FK-506 binding protein (FKBP12)



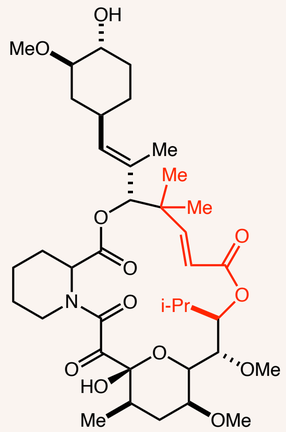
Hypothesis: Inhibition of proline isomerization leads to immunosuppression

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

The proline isomerase hypothesis



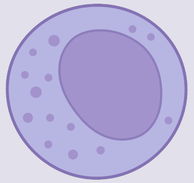
FK-506



506BD

Incredibly potent inhibitor of FKBP12

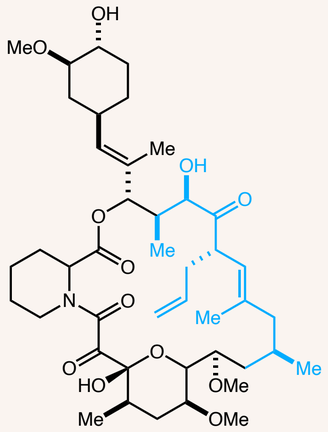
506BD



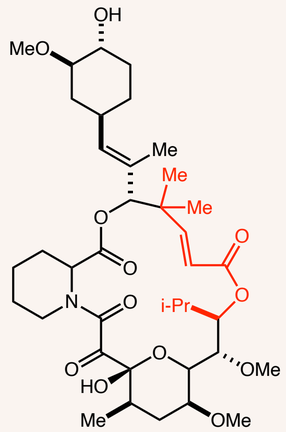
Elicits **no change** in immune phenotype!!!

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

The proline isomerase hypothesis



FK-506



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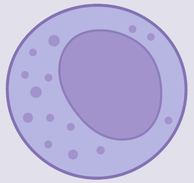

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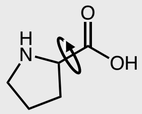


Proline isomerases

Maybe this process is irrelevant to phenotype??

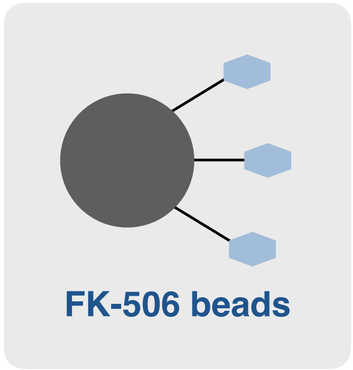
Are there any other proteins involved?

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

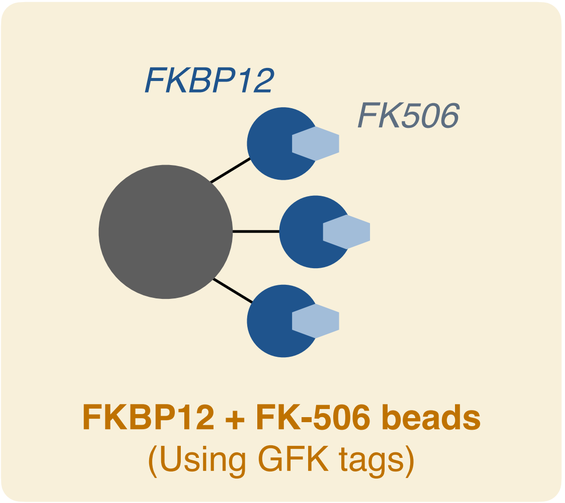
Ternary complex hypothesis

Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66* (4), 807–815.

Ternary complex hypothesis



Initial approach



What proteins bind to the FKBP–FK506 complex?

Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66* (4), 807–815.

Ternary complex hypothesis

Calcineurin Is a Common Target of Cyclophilin-Cyclosporin A and FKBP-FK506 Complexes

Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66* (4), 807–815.

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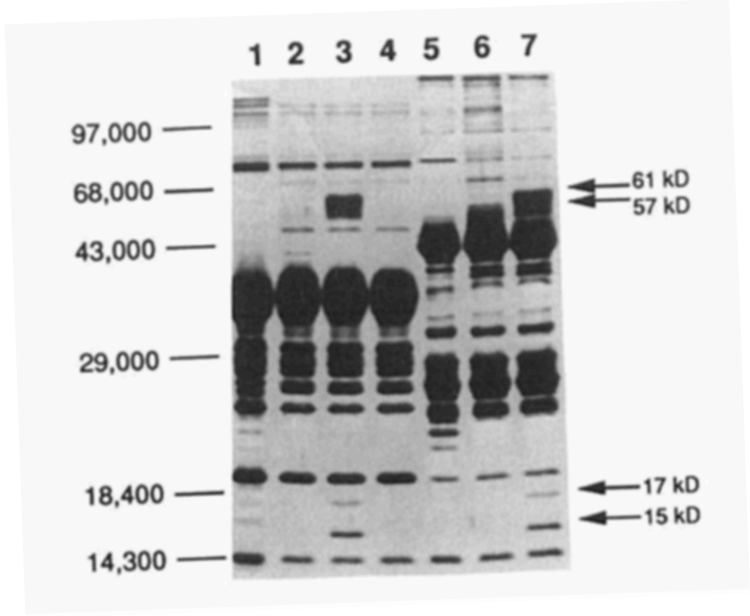

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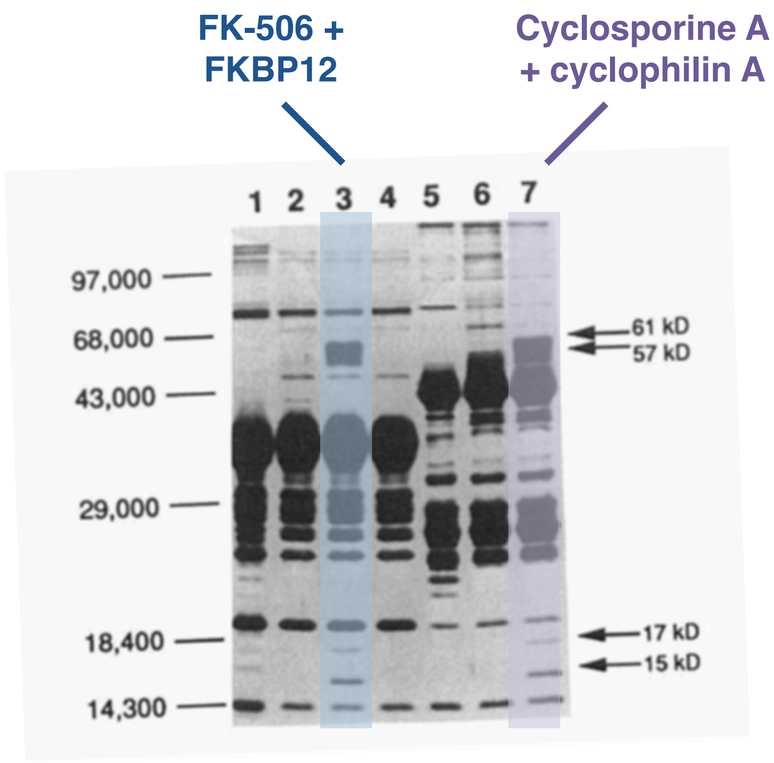

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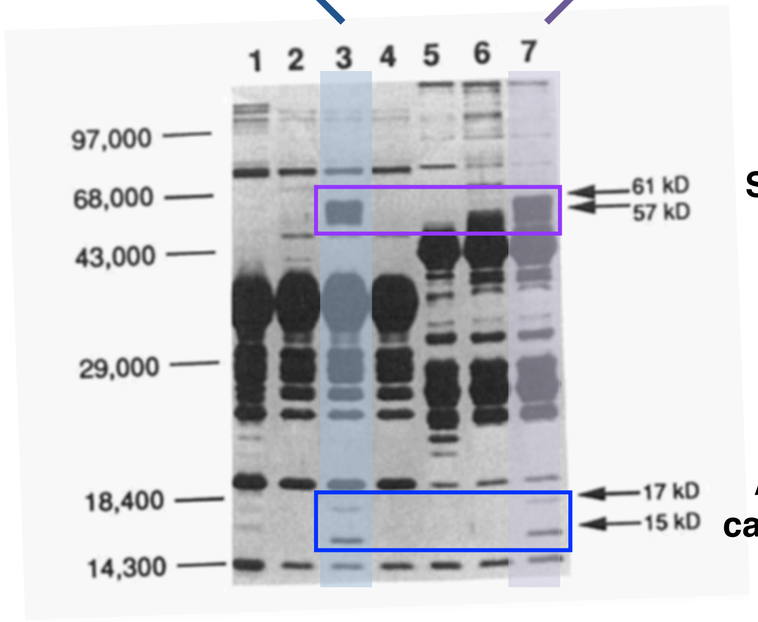
Calcineurin Is a Common Target of Cyclophilin–Cyclosporin A and FKBP–FK506 Complexes

FK-506 + FKBP12

Cyclosporine A + cyclophilin A



Shared band is calcineurin!

Also get weak calmodulin signal

Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66* (4), 807–815.

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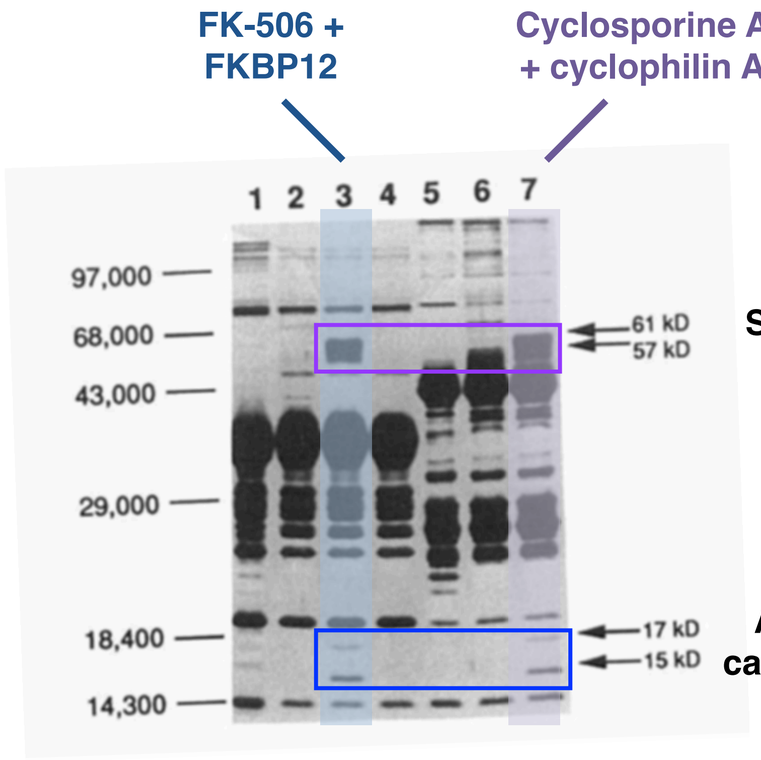

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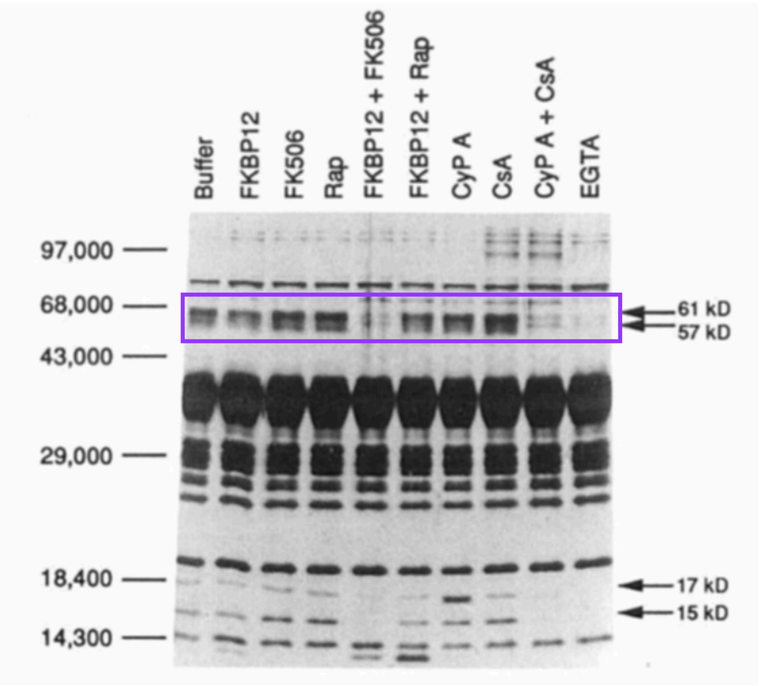

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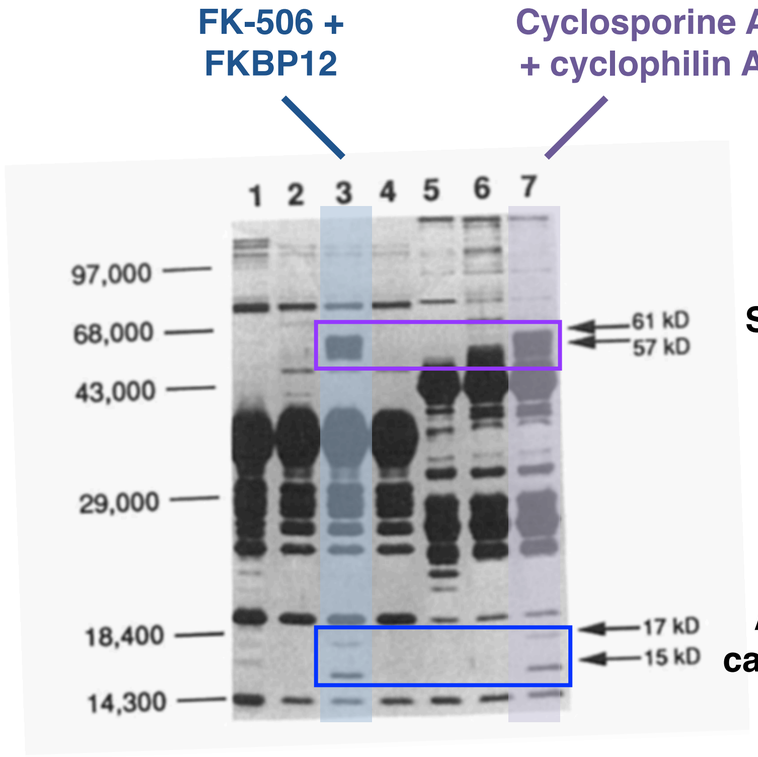

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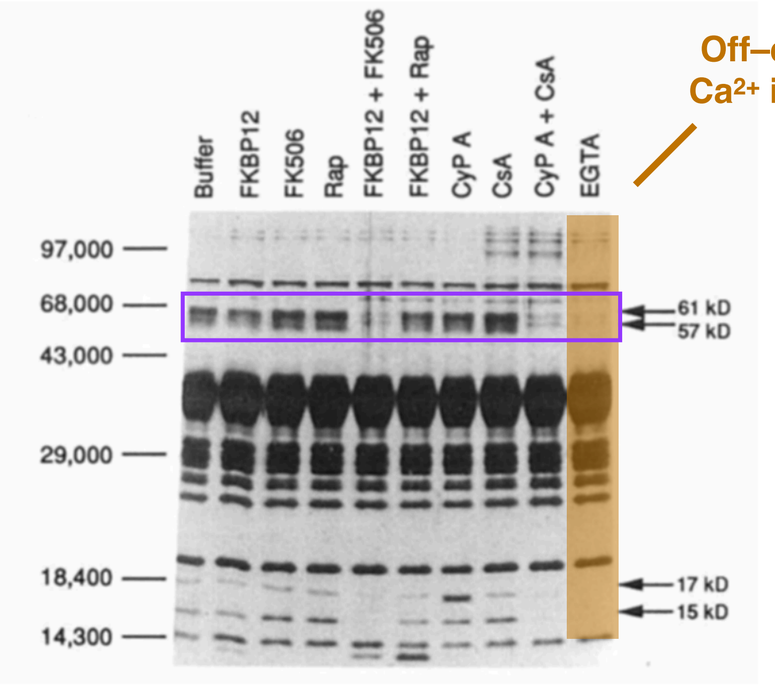
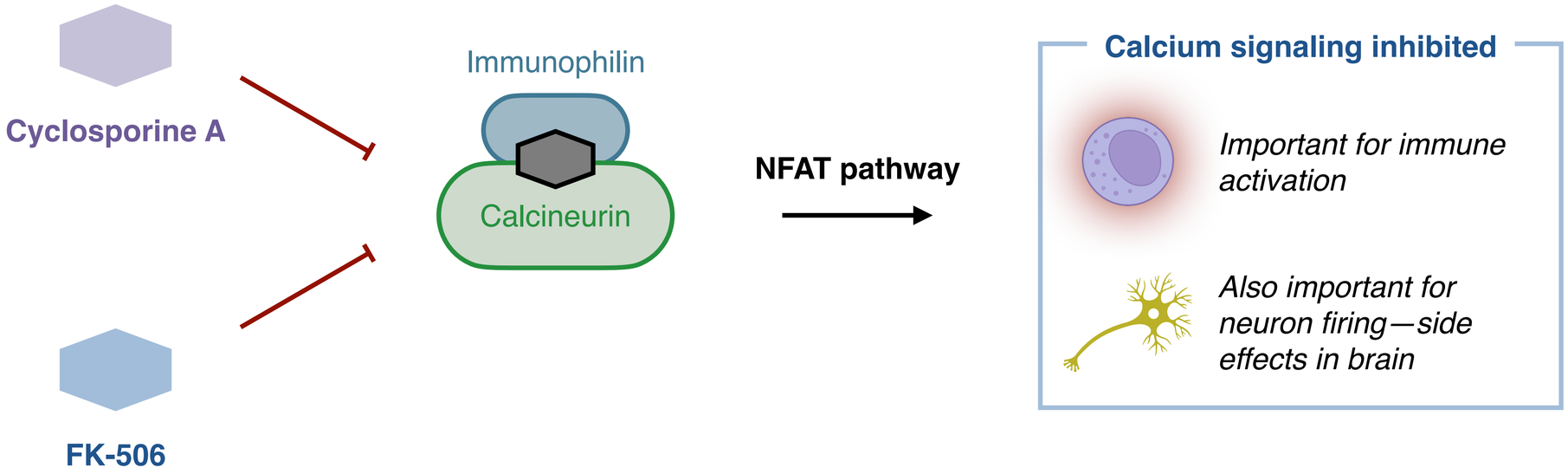


Off–compete with Ca^{2+} ion scavenger

Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66* (4), 807–815.

Why is calcineurin important?



Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66* (4), 807–815.

Ternary complex hypothesis

The mechanism of action of cyclosporin A and FK506

Stuart L. Schreiber and Gerald R. Crabtree

Crabtree

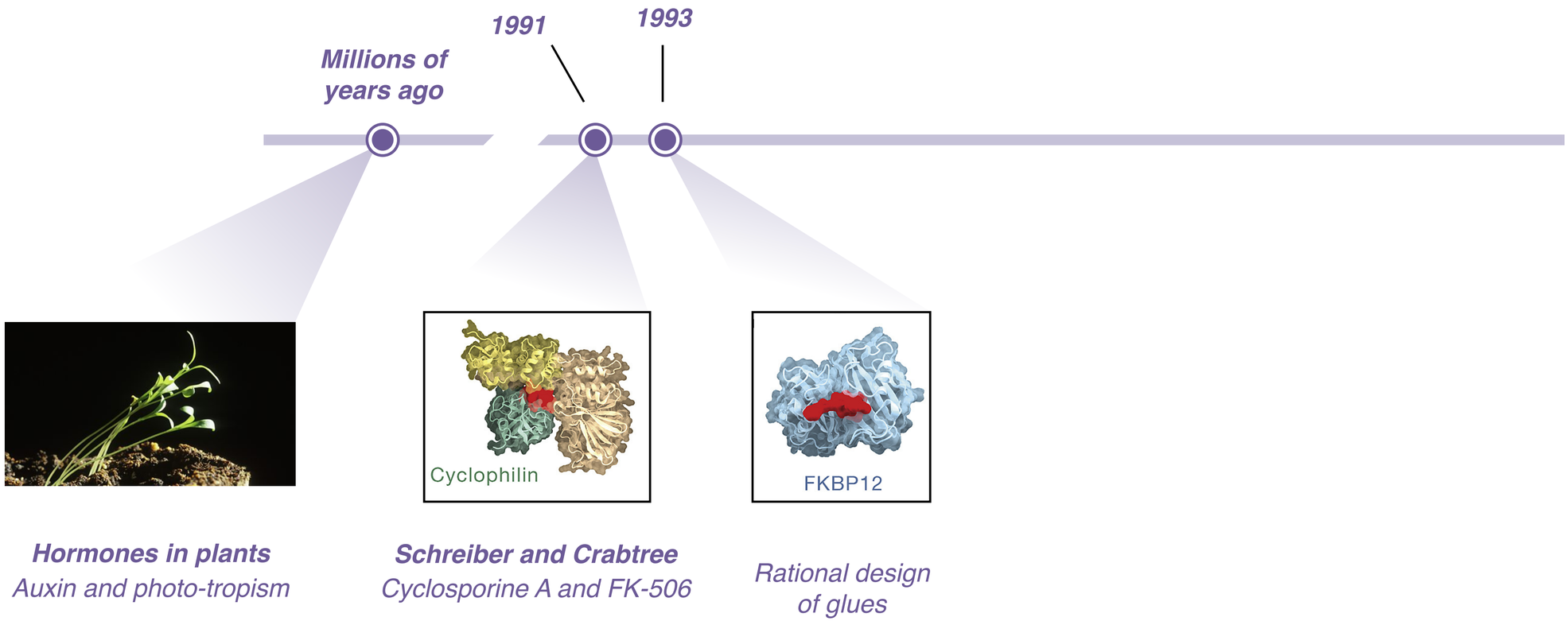
Schreiber

*"…these immunosuppressants exhibit an unprecedented property: they behave like a **'molecular glue'**."*

Ternary complex-inducing drugs can be designed by chemists

Schreiber, S. L.; Crabtree, G. R. *Immunology Today* **1992**, *13* (4), 136–142.

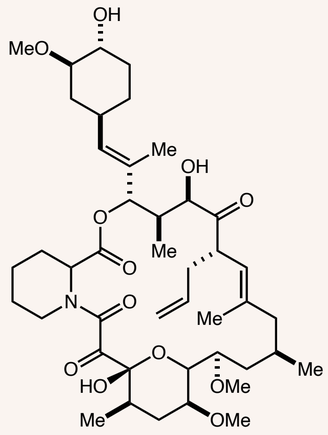
History of induced proximity



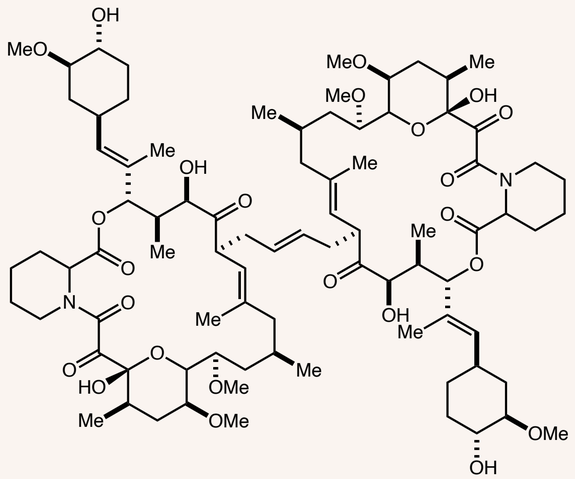
Békés, M.; Langley, D. R.; Crews, C. M. *Nat Rev Drug Discov* **2022**, *21* (3), 181–200.

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

Rationally designed molecular glues



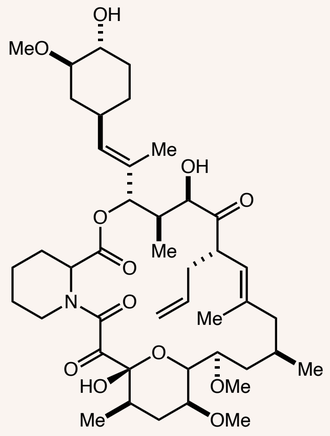
FK-506



FK-1012 (dimer)

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

Rationally designed molecular glues



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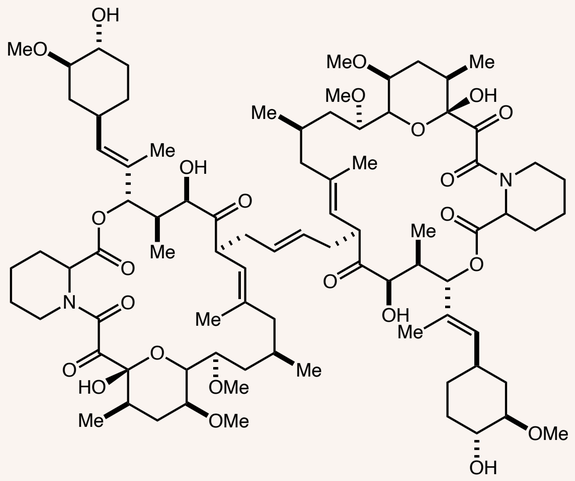
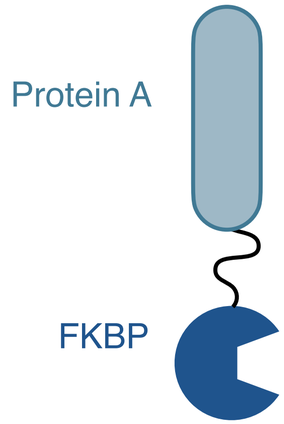


FK-1012 (dimer)

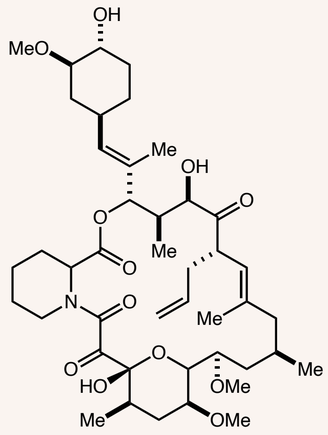
Can induce the dimerization of any protein



Protein A – FKBP fusion

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

Rationally designed molecular glues



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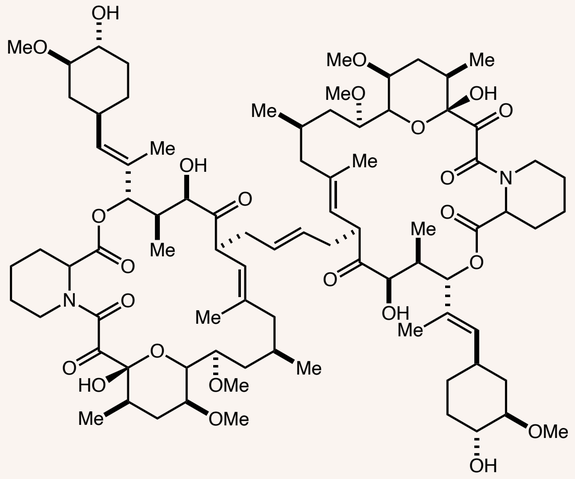

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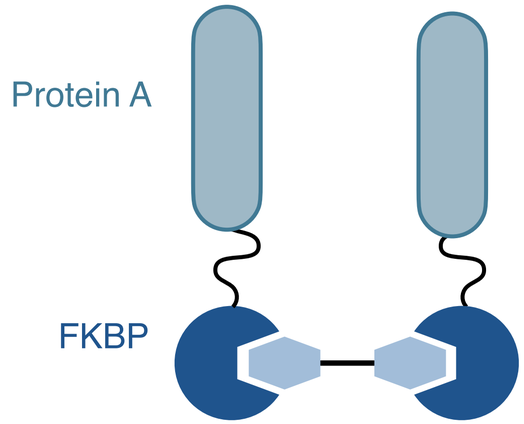
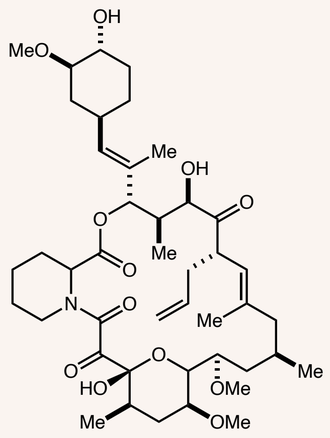


Protein A – FKBP fusion

FK-1012

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

Rationally designed molecular glues



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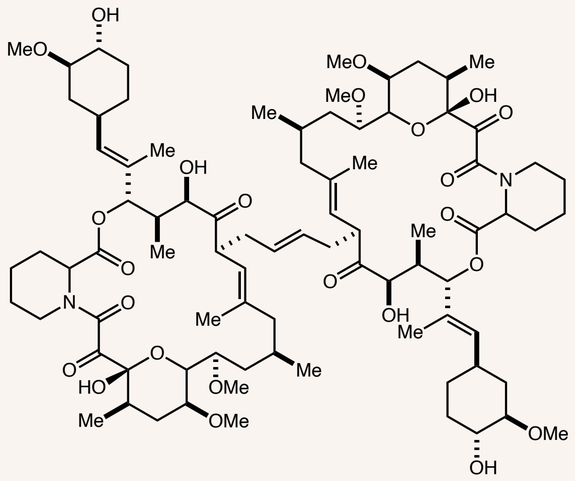

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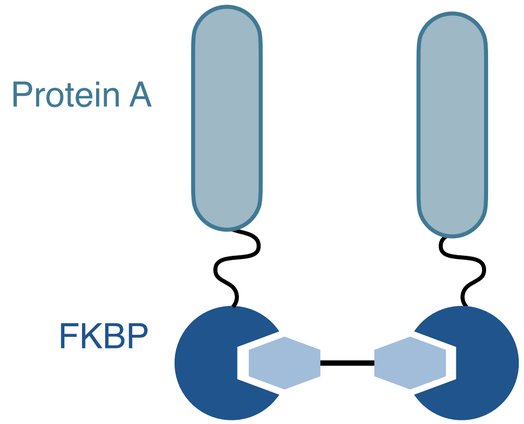


Protein A – FKBP fusion

FK-1012

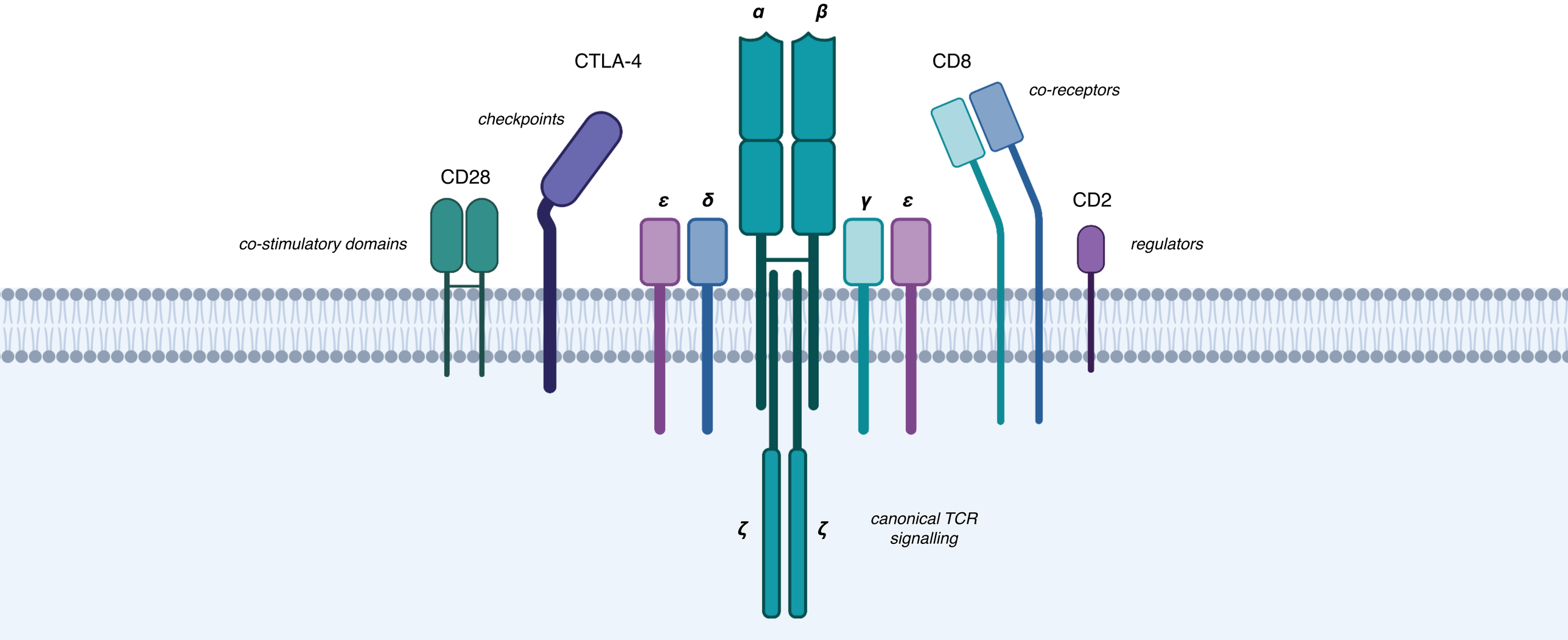
Let's try this on the T-cell receptor!

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

T-Cell signaling requires many key components

T-Cell Receptor (TCR)

relies on complex interactome for proper function



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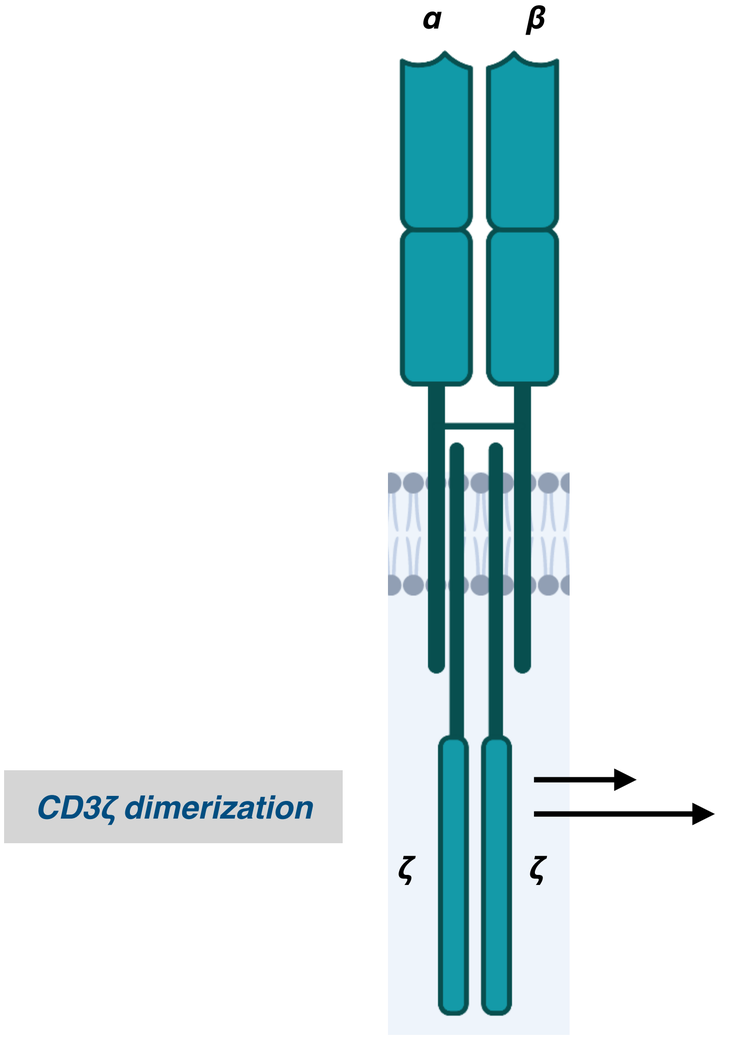

CD3ζ dimerization

Key for downstream signaling and T-cell activation

Can induced dimerization of CD3ζ activate T-cells?

Spencer, D. M.; Wandless, T. J.; Schreiber, S. L.; Crabtree, G. R. *Science* **1993**, *262* (5136), 1019–1024.

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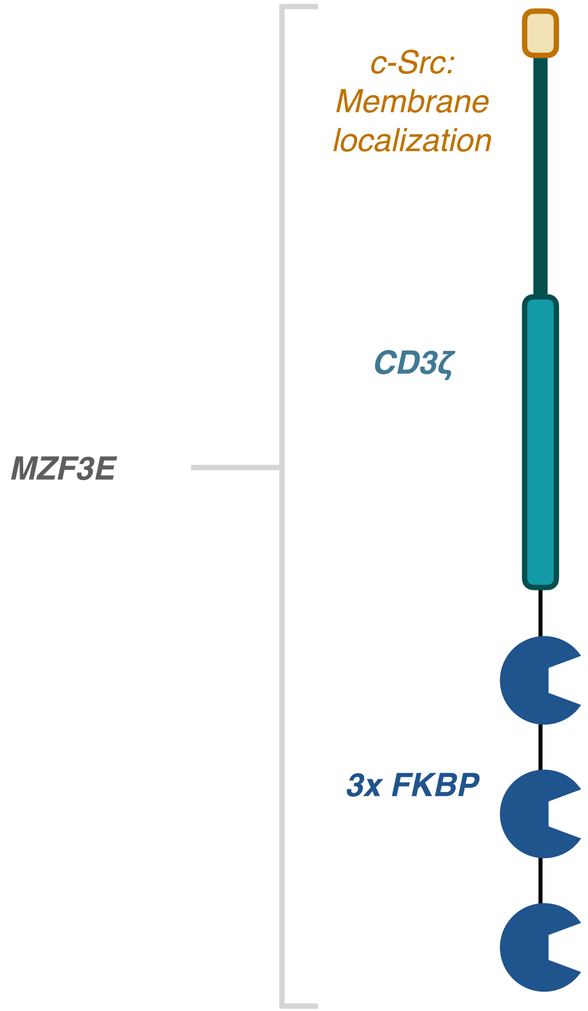

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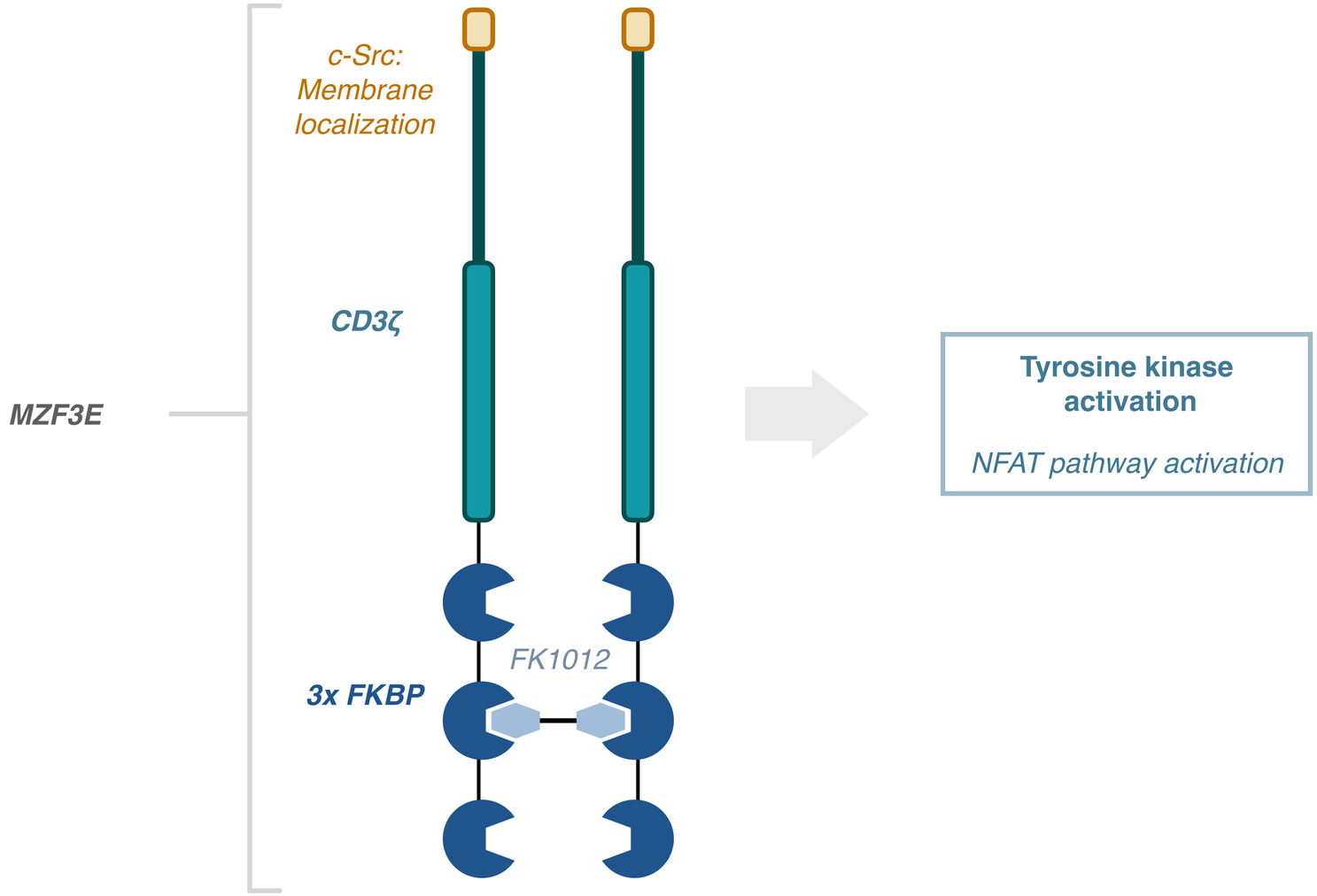

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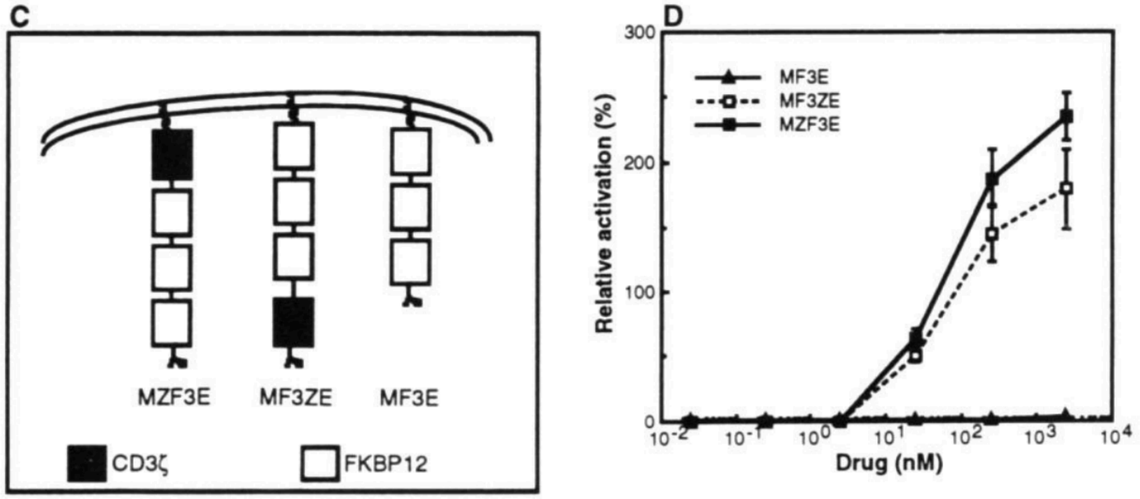

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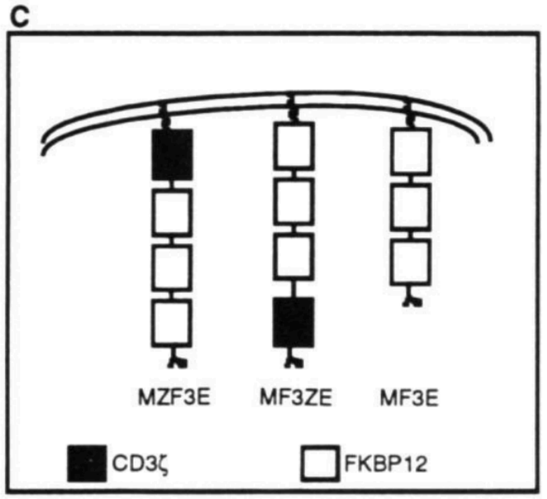

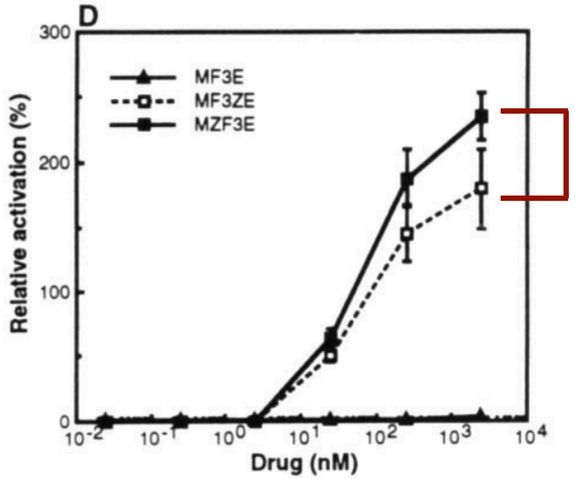

Only CD3 containing constructs produce NFAT response

T-cells are activated!

Spencer, D. M.; Wandless, T. J.; Schreiber, S. L.; Crabtree, G. R. *Science* **1993**, *262* (5136), 1019–1024.

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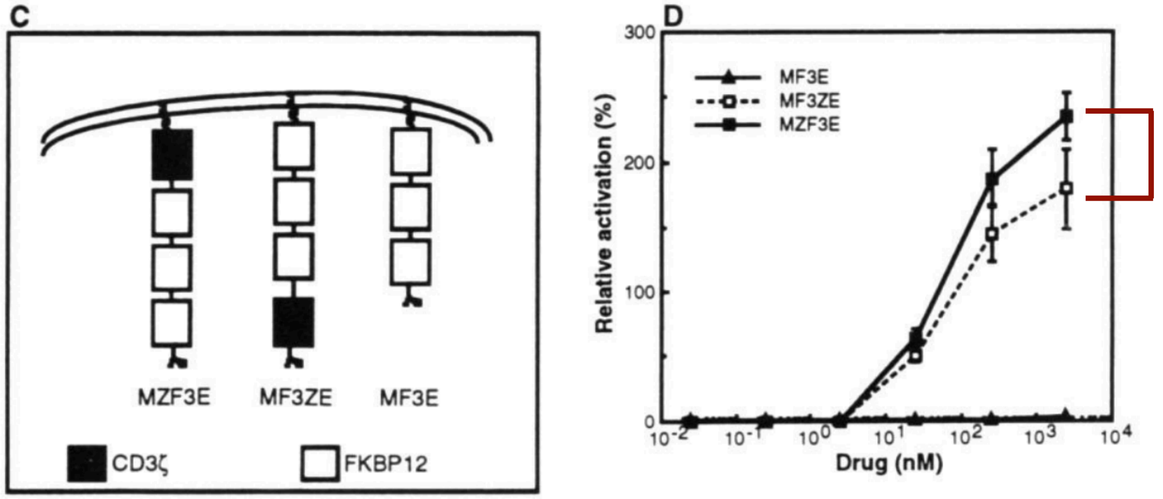


Only CD3 containing constructs produce NFAT response

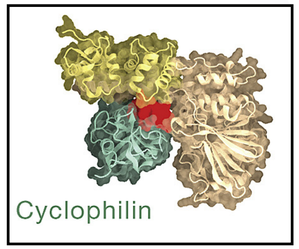
T-cells are activated!

Designed inducers of proximity can produce physiological responses in cells!

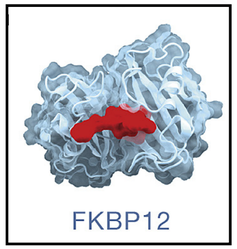
Spencer, D. M.; Wandless, T. J.; Schreiber, S. L.; Crabtree, G. R. *Science* **1993**, *262* (5136), 1019–1024.

History of induced proximity

Hormones in plants
Auxin and photo-tropism



Schreiber and Crabtree
Cyclosporine A and FK-506



Rational design of glues

Békés, M.; Langley, D. R.; Crews, C. M. *Nat Rev Drug Discov* **2022**, *21* (3), 181–200.

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

History of induced proximity

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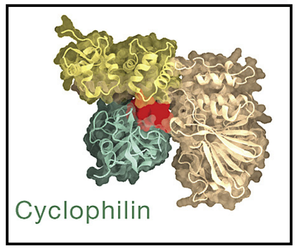

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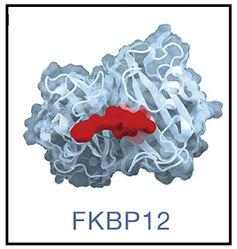

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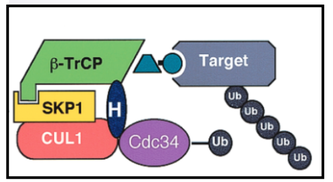


Crews and Deshaies
The first PROTACs

Békés, M.; Langley, D. R.; Crews, C. M. *Nat Rev Drug Discov* **2022**, *21* (3), 181–200.

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

The design of the first PROTACs

Craig Crews

Raymond Deshaies

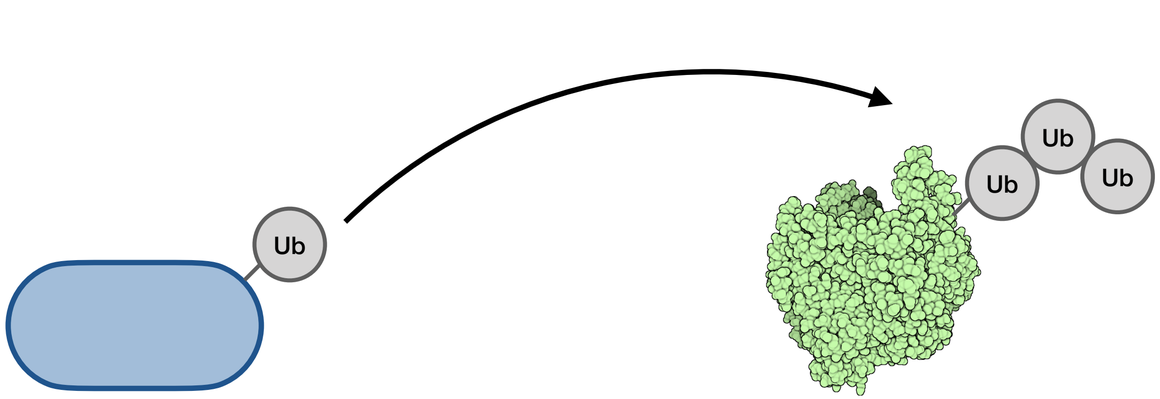
Can we leverage induced proximity outside the context of FKBP12?

Both labs focused on the 26S proteasome

How the majority of proteins are degraded in the cell

26S Proteasome

The design of the first PROTACs

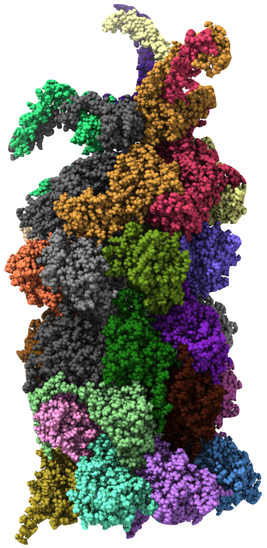


E3 ligase

Targets proteins for ubiquitination

Ubiquitinated protein

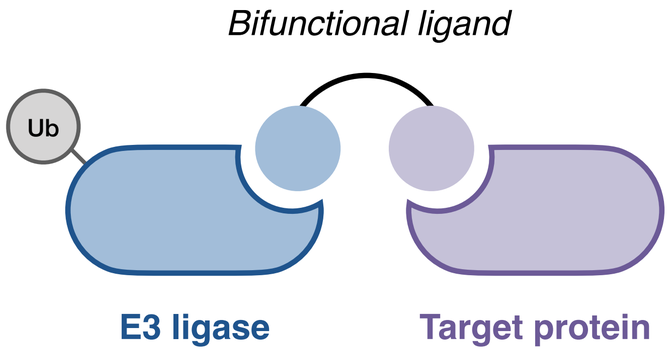
Tagged for degradation



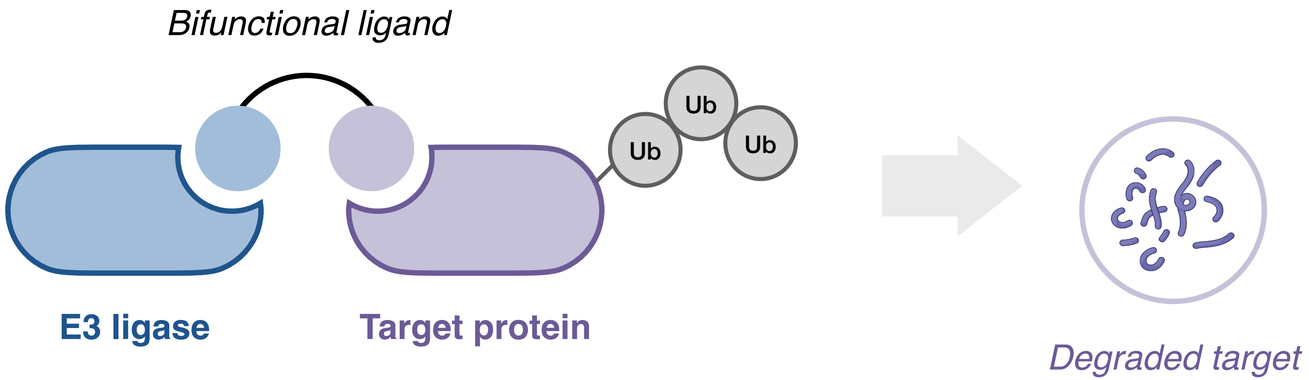
26S Proteasome

Leveraging E3 ligases for induced proximity platforms

Leveraging E3 ligases for induced proximity platforms



Leveraging E3 ligases for induced proximity platforms



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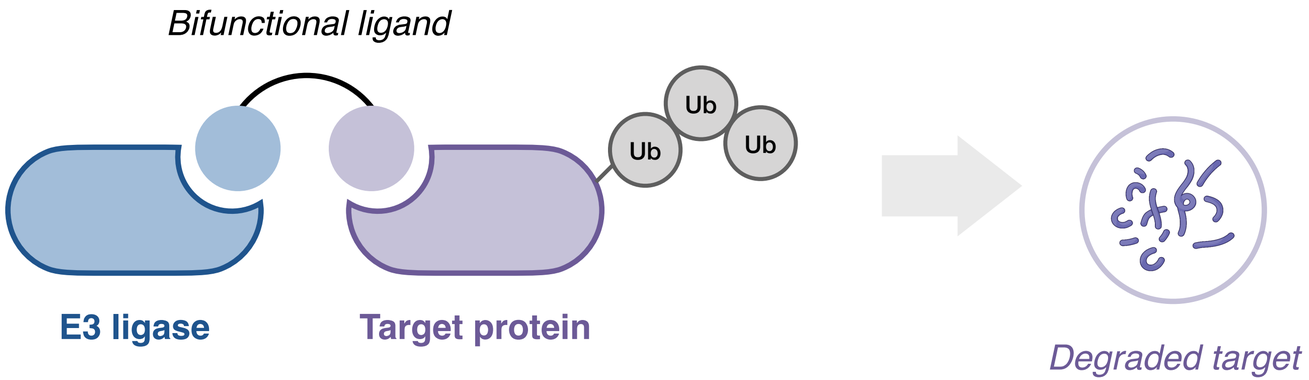
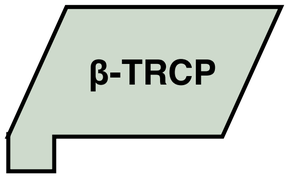


PROteolysis **TA**rgeting **C**himeras

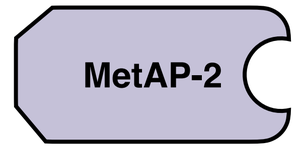
Can theoretically degrade any protein

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

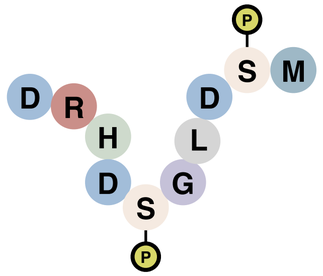
Leveraging E3 ligases for induced proximity platforms



E3 ligase

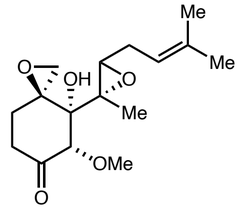


Target



IPP

*Phospho-peptide recognition sequence (**degron**)*

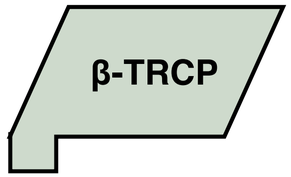


OVA

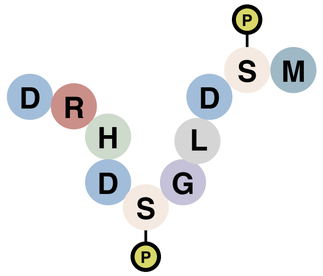
Ovalicin (Inhibitor of Met-AP-2)

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

Leveraging E3 ligases for induced proximity platforms



E3 ligase



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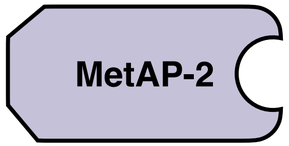

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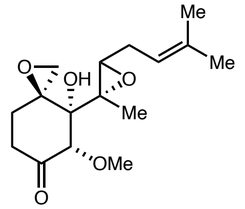
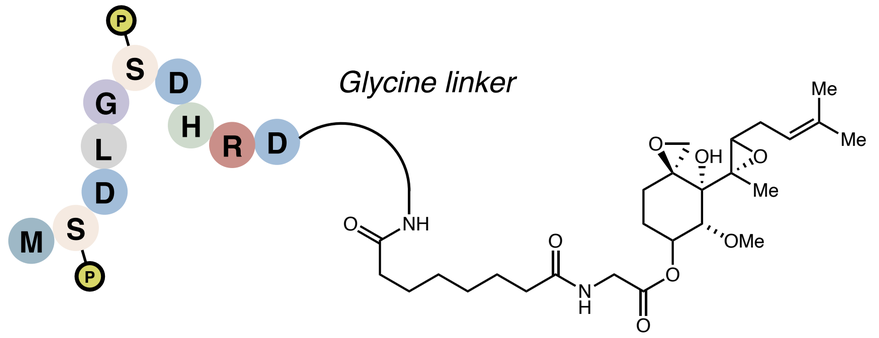


OVA

Ovalicin (Inhibitor of Met-AP-2)

PROTAC-1



Should degrade MetAP-2

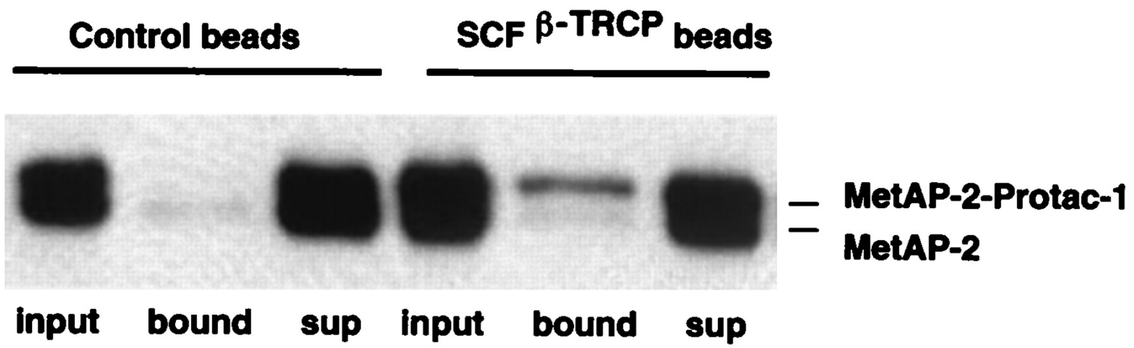
Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

PROTAC-1 binding and ubiquitination

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

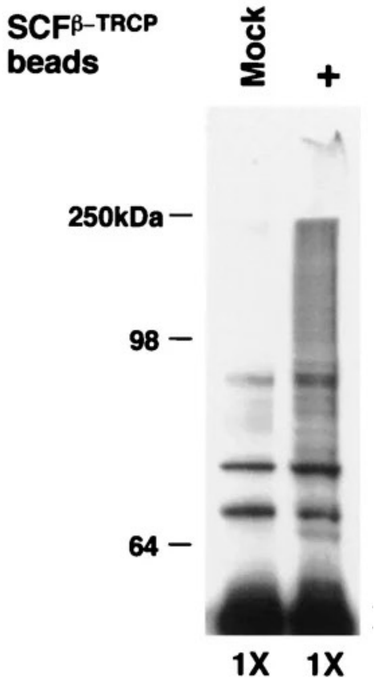
PROTAC-1 binding and ubiquitination

Immunoprecipitation



PROTAC-1 induces binding of MetAP-2

Ubiquitination



PROTAC-1 induces ubiqutination of MetAP-2

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

Does PROTAC-1 degrade MetAP-2 in “endogenous contexts”?

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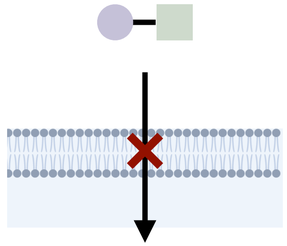
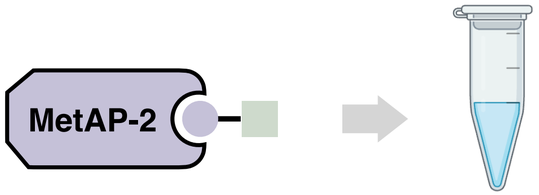
Does PROTAC-1 degrade MetAP-2 in "endogenous contexts"?

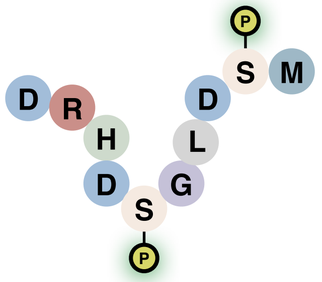
Challenges of a degradation study



PROTAC-1 is impermeable



Recombinant MetAP-2 + PROTAC added to cell extract



PROTAC-1 needs to be constitutively phosphorylated

Recombinant kinase and small molecule phosphatase inhibitor added to extract

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

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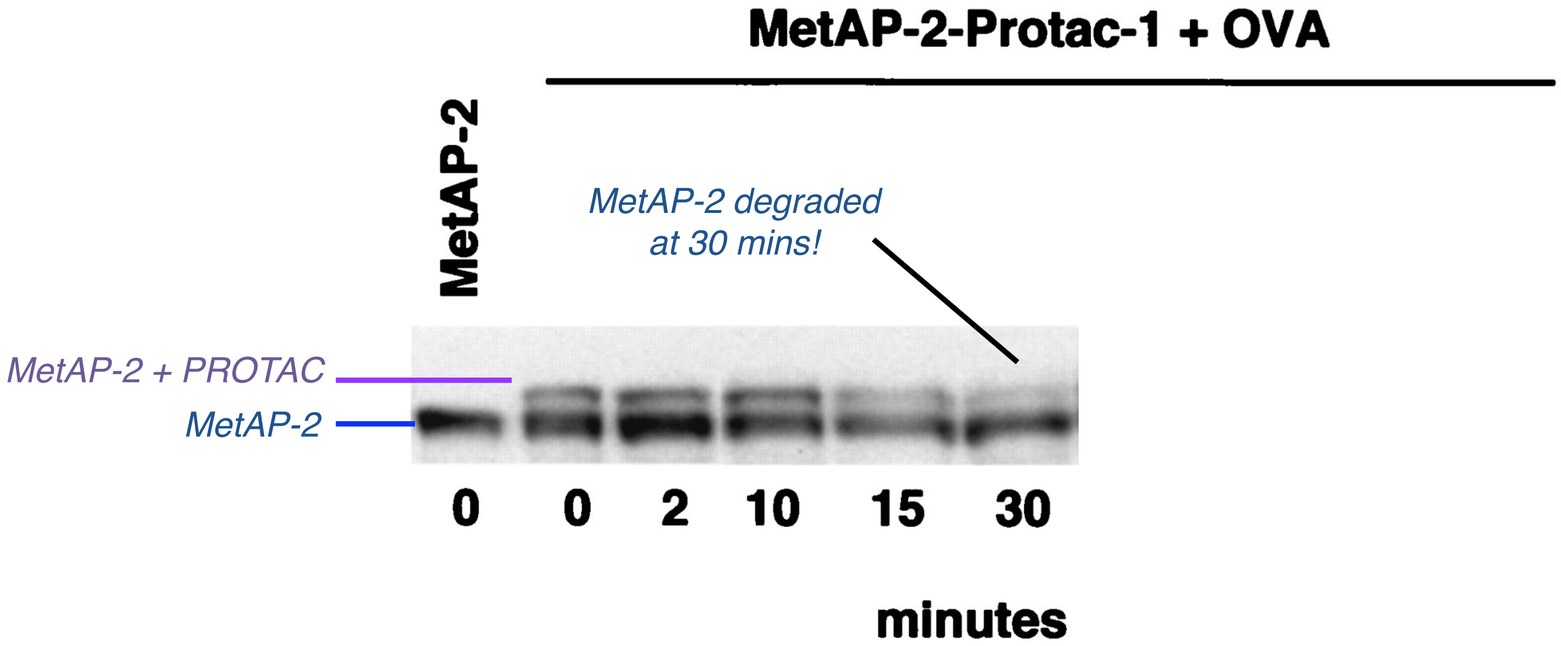

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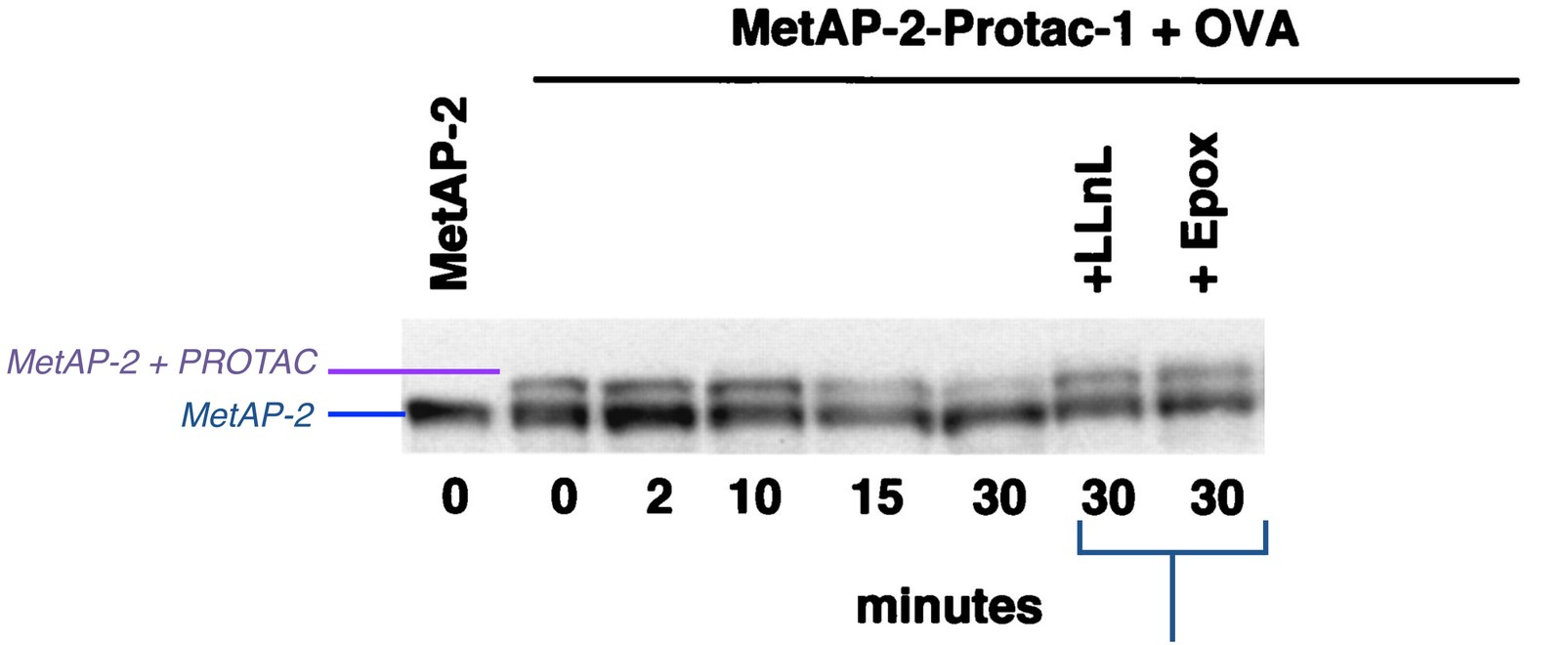
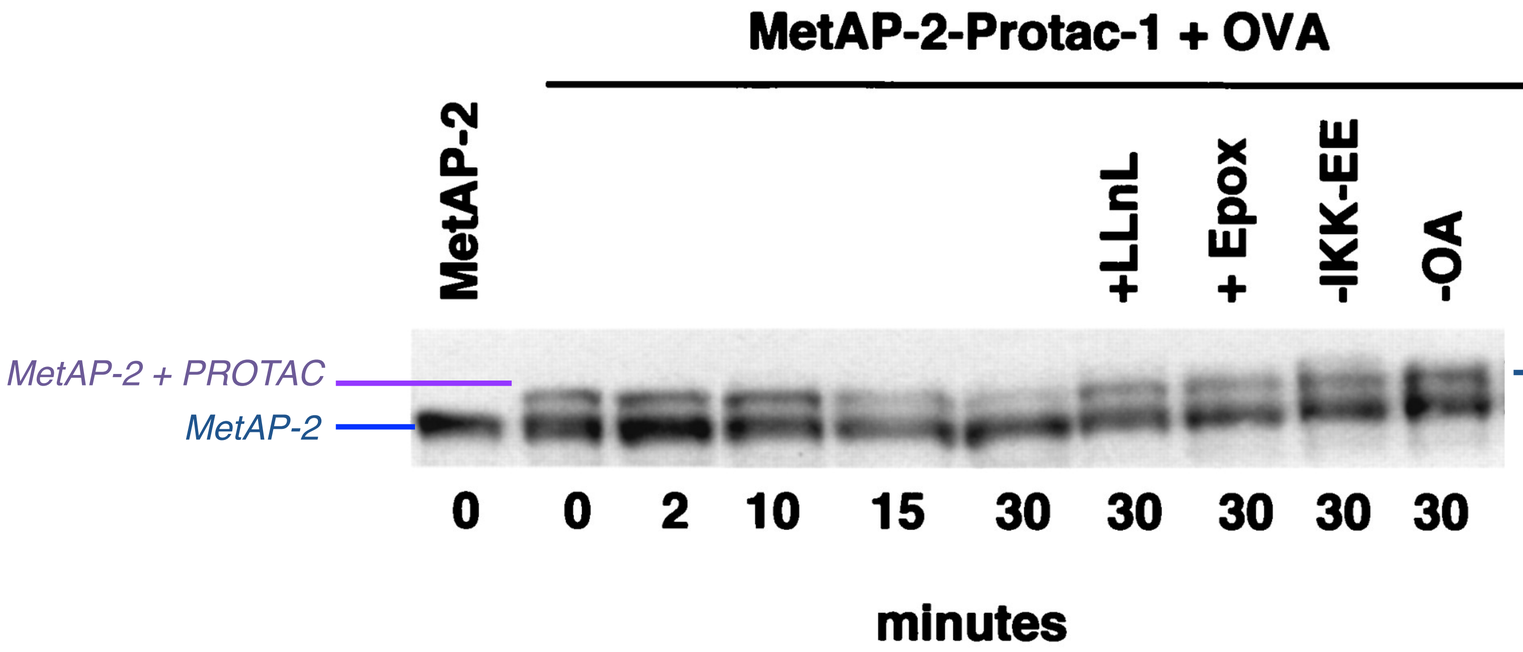
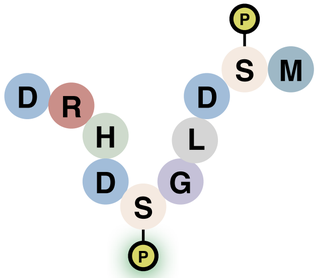


Proteasome inhibitors rescue degradation

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

Does PROTAC-1 degrade MetAP-2 in “endogenous contexts”?





PROTAC-1 needs to be constitutively phosphorylated

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

Implications of PROTACs

Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

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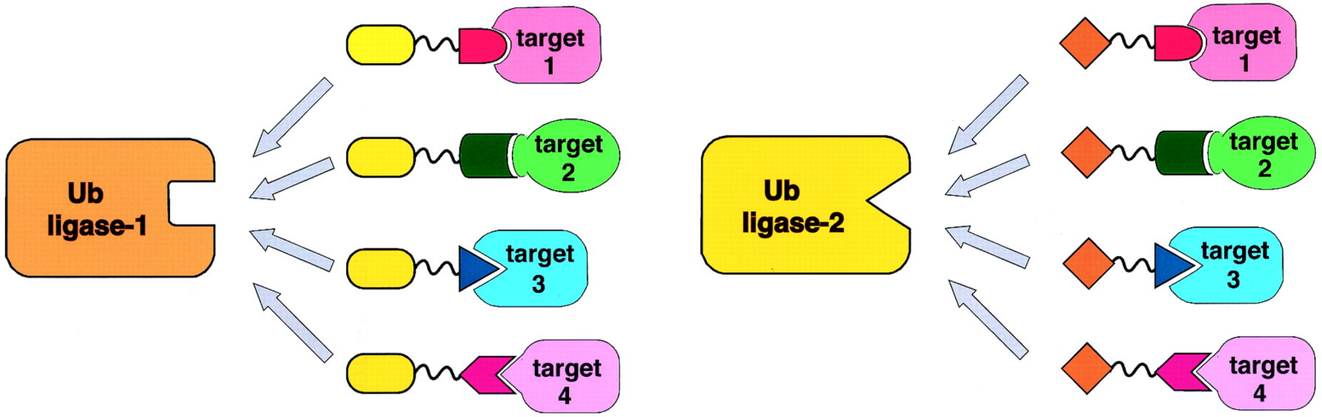


“We envision the potential to develop an entire suite of Protac compounds…”

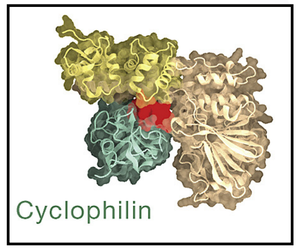
PROTACs are interesting, but not useful at this stage

- Membrane impermeable
- Require extensive phosphatase inhibition

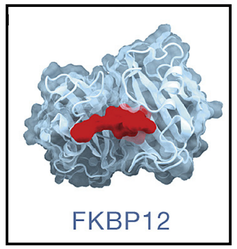
Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *PNAS* **2001**, *98* (15), 8554–8559.

History of induced proximity

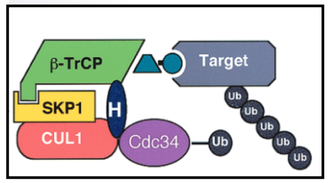
Hormones in plants
Auxin and photo-tropism



Schreiber and Crabtree
Cyclosporine A and FK-506



Rational design of glues



Crews and Deshaies
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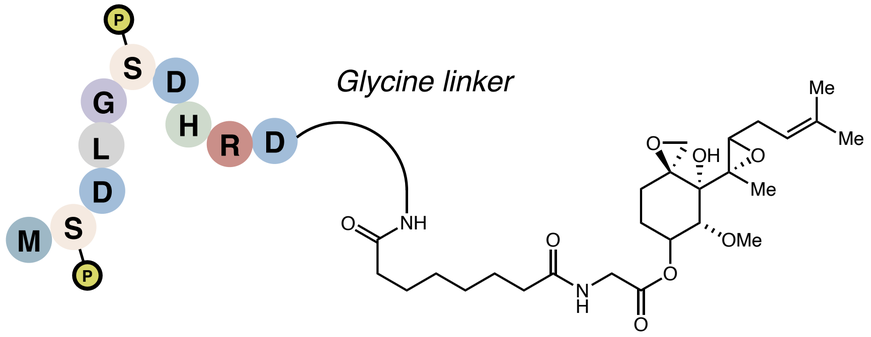
Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

The extensive undertaking of making PROTACs usable

Crews Lab

PROTAC-1



- Needs to be membrane permeable
- Needs to be stable in the cell

Entirely small molecule PROTAC

How can we optimize PROTAC-1?

The extensive undertaking of making PROTACs usable

2008

Targeted intracellular protein degradation induced by a small molecule: En route to chemical proteomics

Ashley R. Schneekloth [a], Mathieu Pucheault [b,†], Hyun Seop Tae [b], Craig M. Crews [a,b,c,*]

Schneekloth, A. R.; Pucheault, M.; Tae, H. S.; Crews, C. M. *Bioorganic & Medicinal Chemistry Letters* **2008**, *18* (22), 5904–5908.

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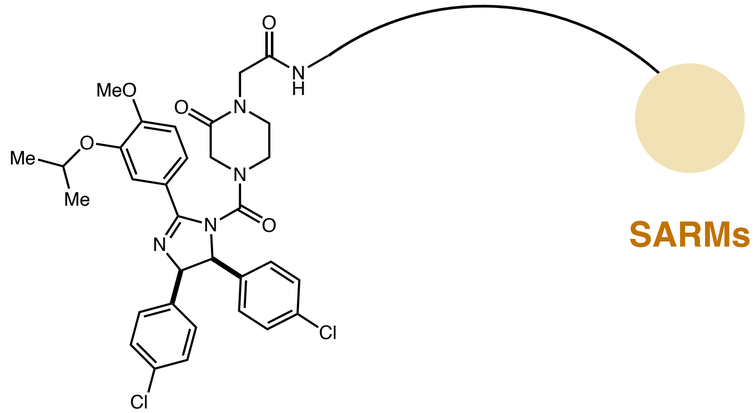
SARM-nutlin PROTAC

MDM2

E3 ligase



SARMs

Nutlin analogue

AR

Androgen receptor

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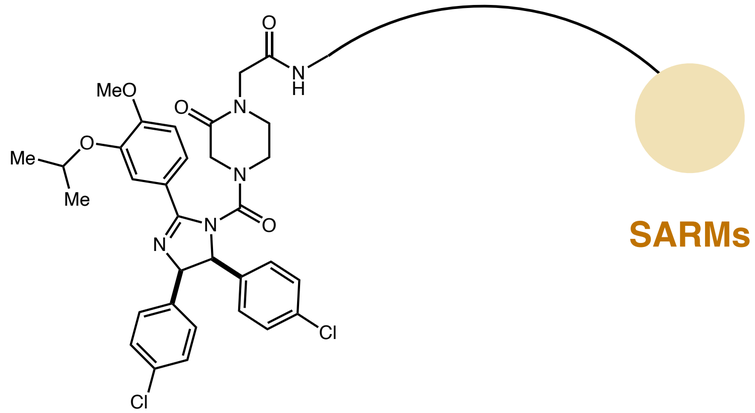

SARMs

AR

Androgen receptor

Nutlin analogue

Cell permeable ✓ **Stable in cells** ✓

BUT

Requires very high concentrations of PROTAC ✗

Schneekloth, A. R.; Pucheault, M.; Tae, H. S.; Crews, C. M. *Bioorganic & Medicinal Chemistry Letters* **2008**, *18* (22), 5904–5908.

Targeting the VHL E3 ligase

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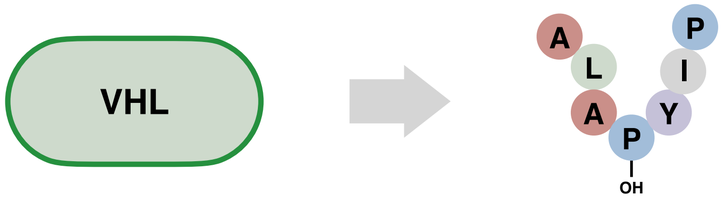
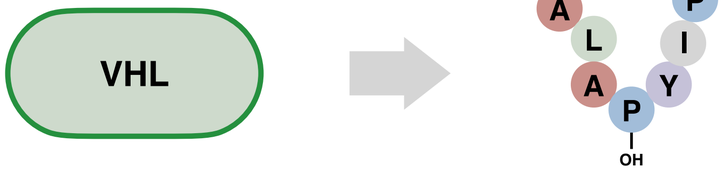


Recognizes and degrades proteins with 7mer peptide sequence with central ***hydroxyproline***

Targeting the VHL E3 ligase



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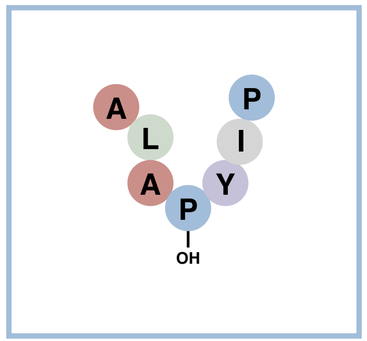
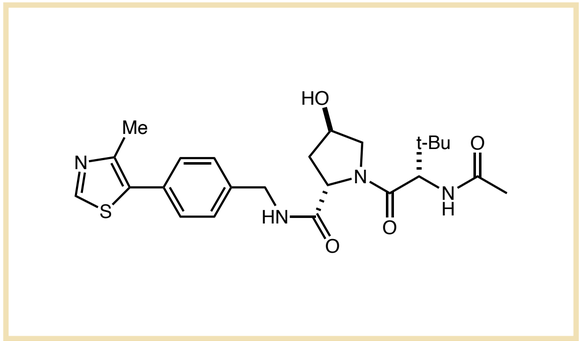




2004 AR degraders

25–100 μM

Schneekloth, J. S. et. al.. *J. Am. Chem. Soc.* **2004**, *126* (12), 3748–3754.

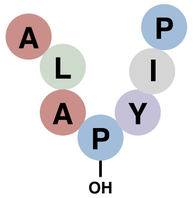
2015 RIPK2 degraders

1–30 nM

Bondeson, D. P. et. al. *Nat Chem Biol* **2015**, *11* (8), 611–617.

Targeting the VHL E3 ligase

VHL



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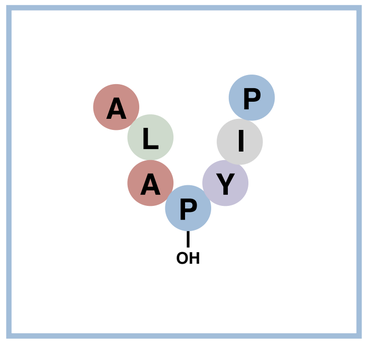

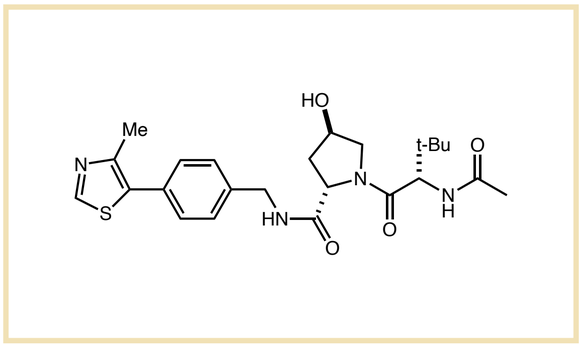

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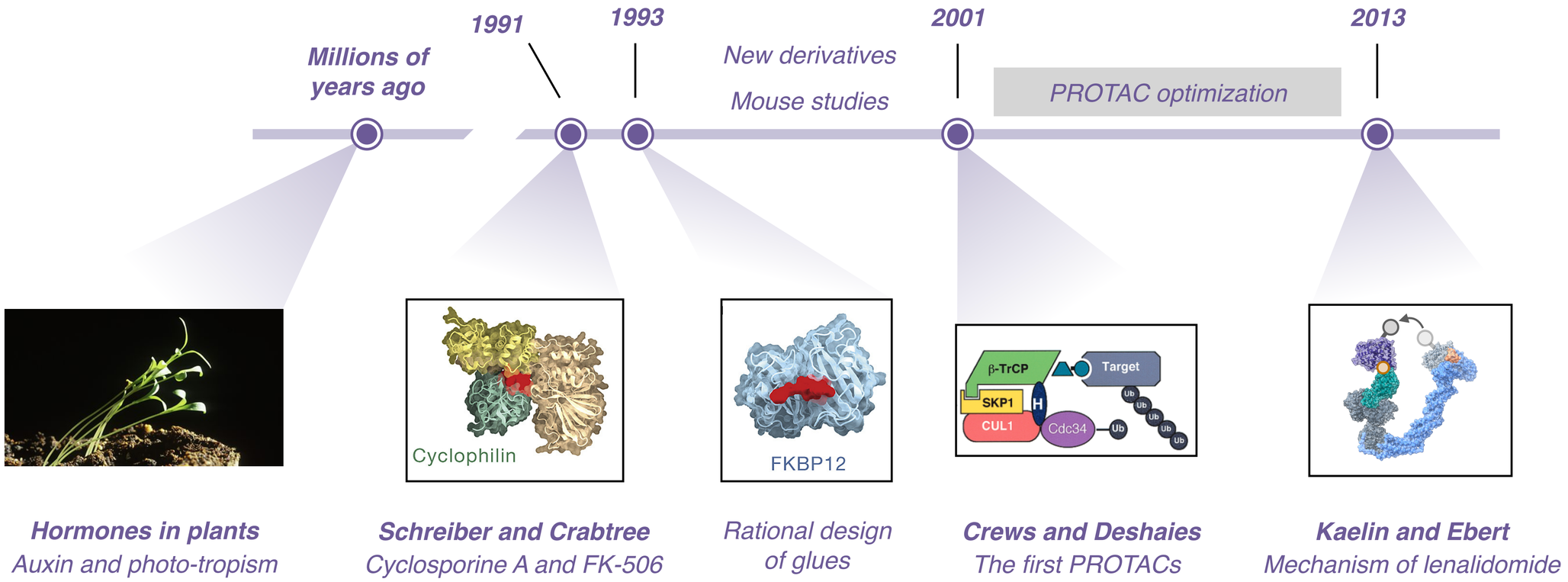
After 10+ years of optimization, VHL PROTACs became incredibly potent degraders

History of induced proximity



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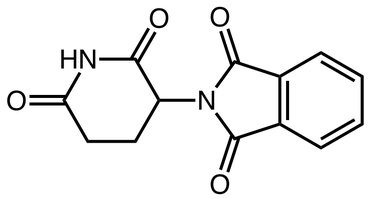
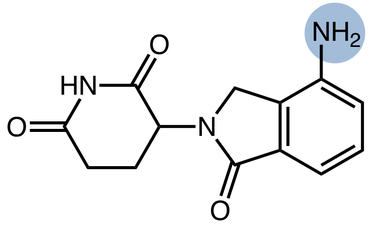
37 YEARS LATER,
A SECOND CHANCE
FOR THALIDOMIDE

New York Times, 1997



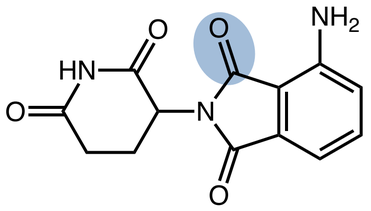
Thalidomide

Approved for leprosy, 1997



Lenalidomide

$10.1 billion in sales (2022)



Pomalidomide

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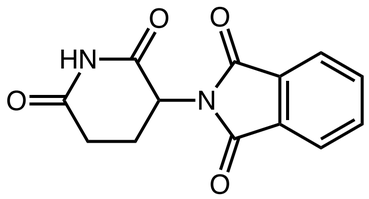

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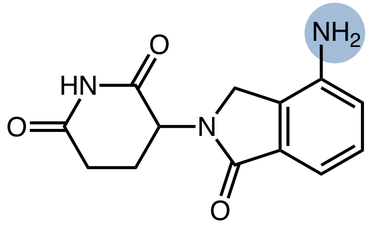

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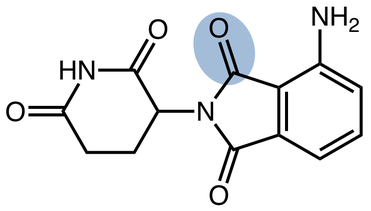

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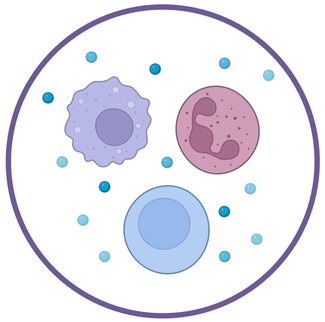
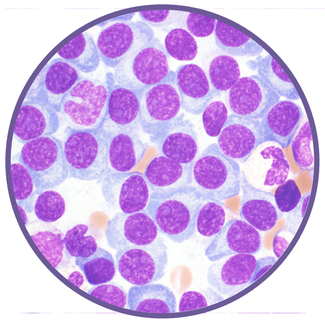
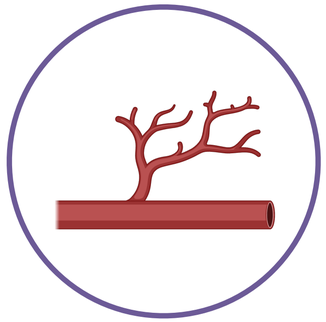


Immune activation



Myeloma cell death



Angiogenesis reduction

Array of additional unique phenotypes

Mechanism unknown for > 15 years!

*IMiDs are **Molecular Glues** that Induce Targeted Protein Degradation*

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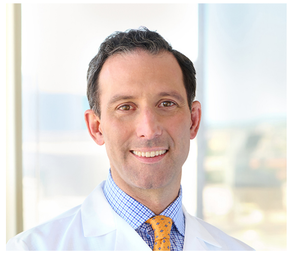
Dana-Farber Cancer Institute



Ben Ebert

Bill Kaelin

2013

Lenalidomide Causes Selective Degradation of IKZF1 and IKZF3 in Multiple Myeloma Cells

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The Myeloma Drug Lenalidomide Promotes the Cereblon-Dependent Destruction of Ikaros Proteins

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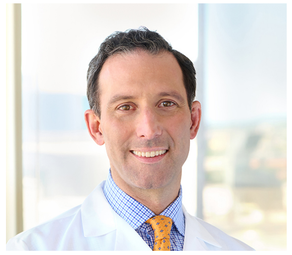

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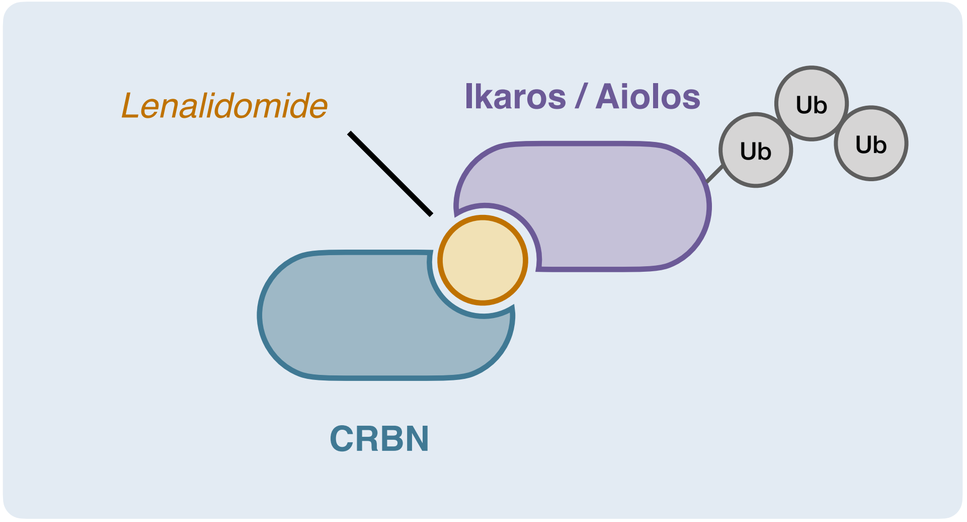
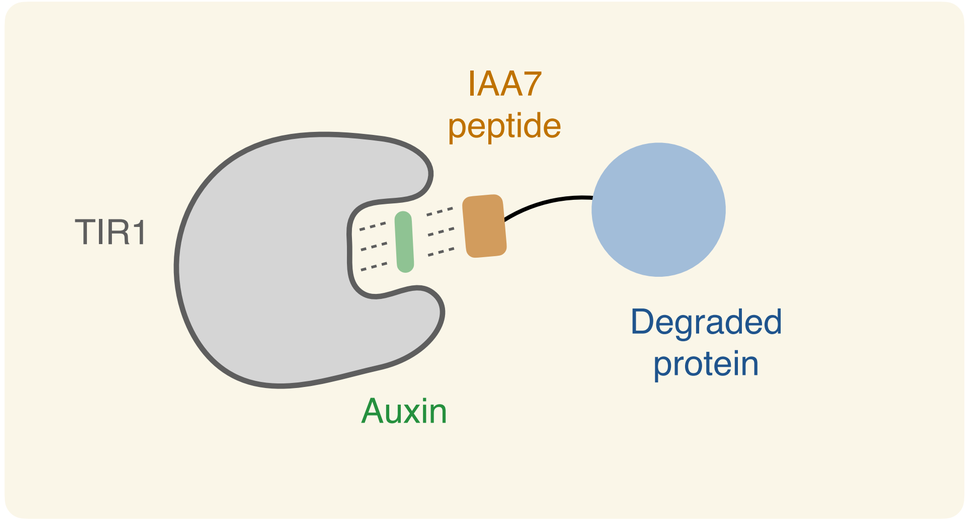


Reminiscent of…



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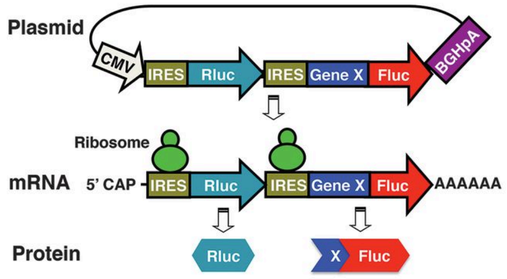
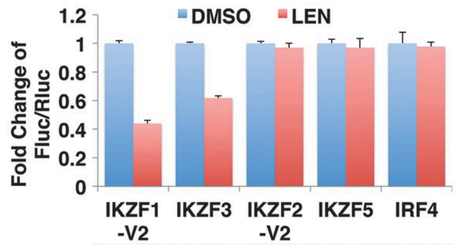
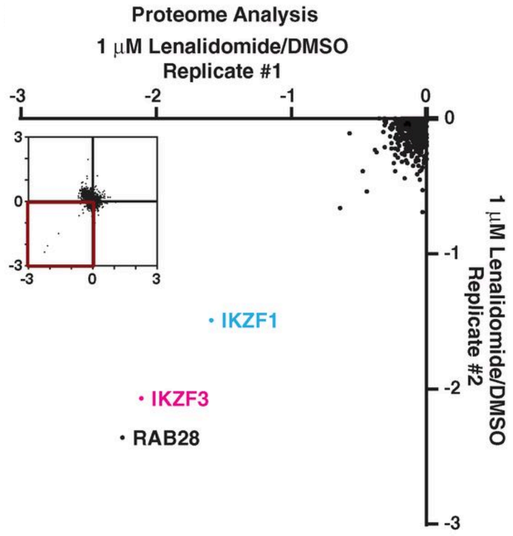




Kaelin Lab

Genetic library of >15,000 plasmids



Ebert Lab

Proteomics approach

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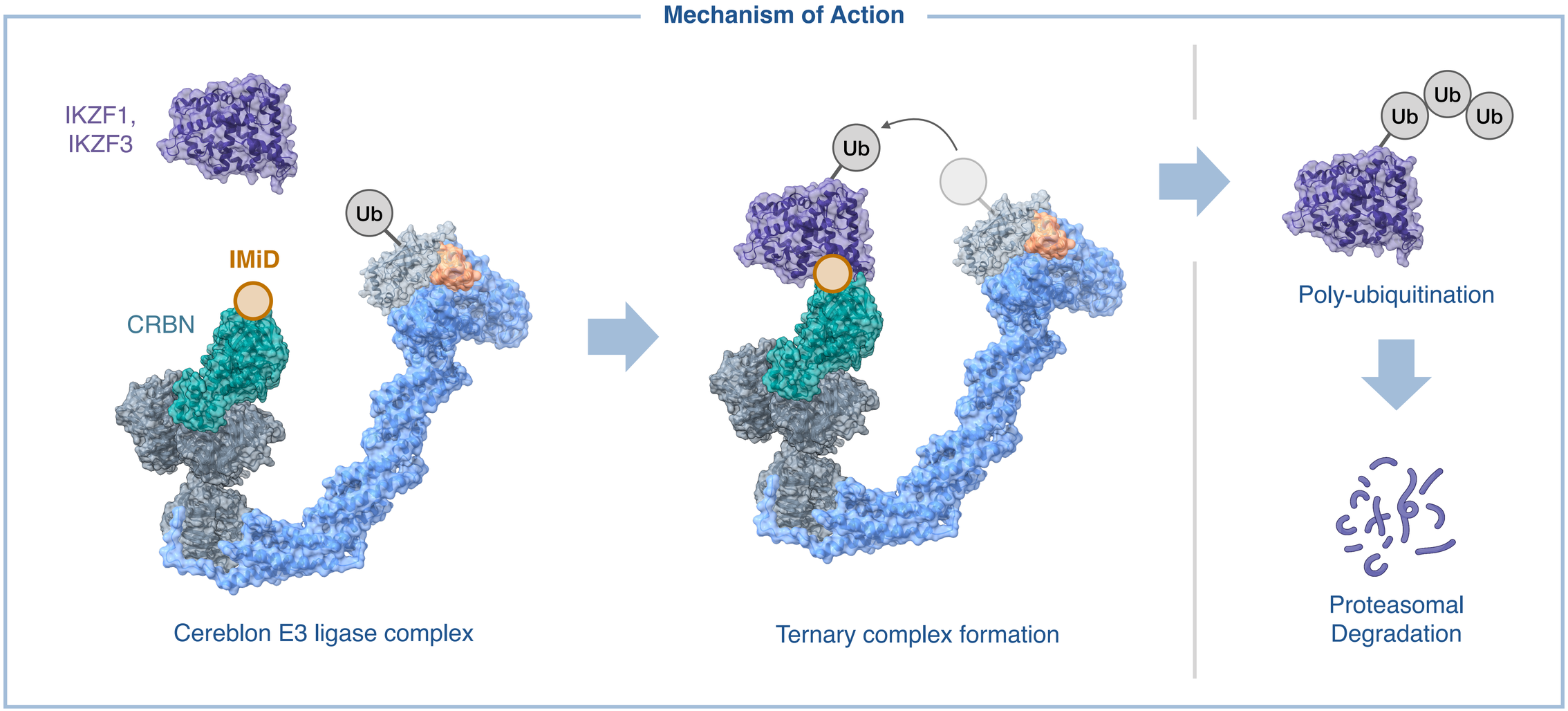

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Conclusions of mechanistic understanding of lenalidomide

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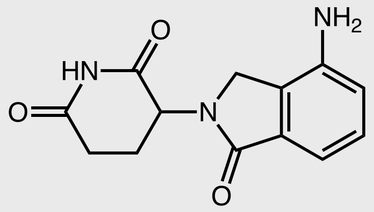
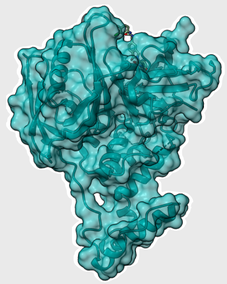
1 *Incredibly small molecules designed by chemists can induce ternary complexes*



***Molecular glues** may offer advantages over linker-based modalities like PROTACs*

2 ***Cereblon** is a robust E3 ligase that can be leveraged for **targeted protein degradation***



Can CRBN be used to degrade any other proteins?

Utilizing CRBN for targeted protein degradation

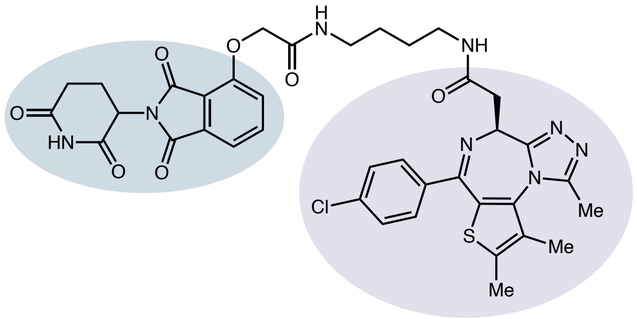
Utilizing CRBN for targeted protein degradation

Dana-Farber Cancer Institute

Georg Winter

Jay Bradner

dBET1



Pomalidomide
CRBN

JQ1
BRD4

BRD4

Winter, G. E.; Buckley, D. L.; Paulk, J.; Roberts, J. M.; Souza, A.; Dhe-Paganon, S.; Bradner, J. E. *Science* **2015**, *348* (6241), 1376–1381.

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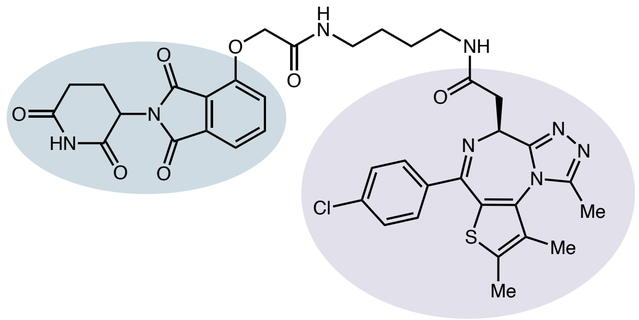
Georg Winter

Jay Bradner

dBET1



Pomalidomide
CRBN

JQ1
BRD4

BRD4

Degrades BRD4 incredibly well!

CRBN can be used in targeted protein degradation modalities

Winter, G. E.; Buckley, D. L.; Paulk, J.; Roberts, J. M.; Souza, A.; Dhe-Paganon, S.; Bradner, J. E. *Science* **2015**, *348* (6241), 1376–1381.

Golden age of induced proximity (2015–present)

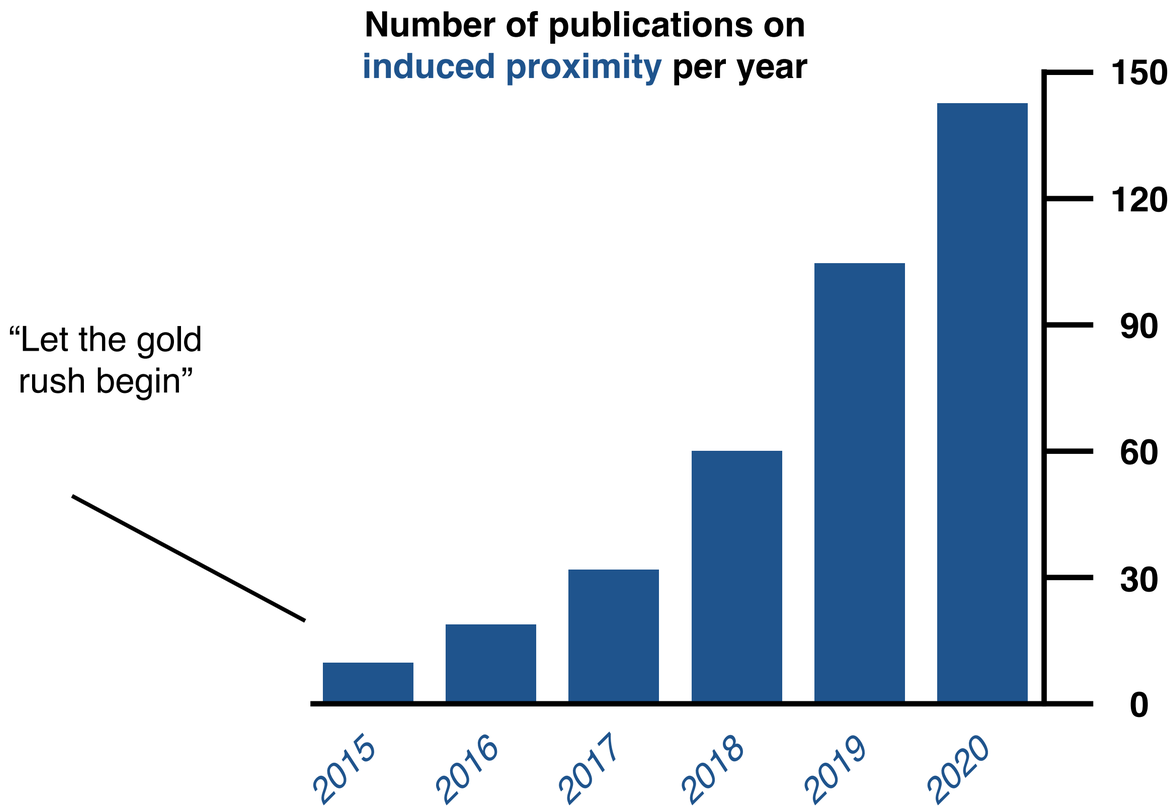
Exponential growth in induced proximity publications

Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.

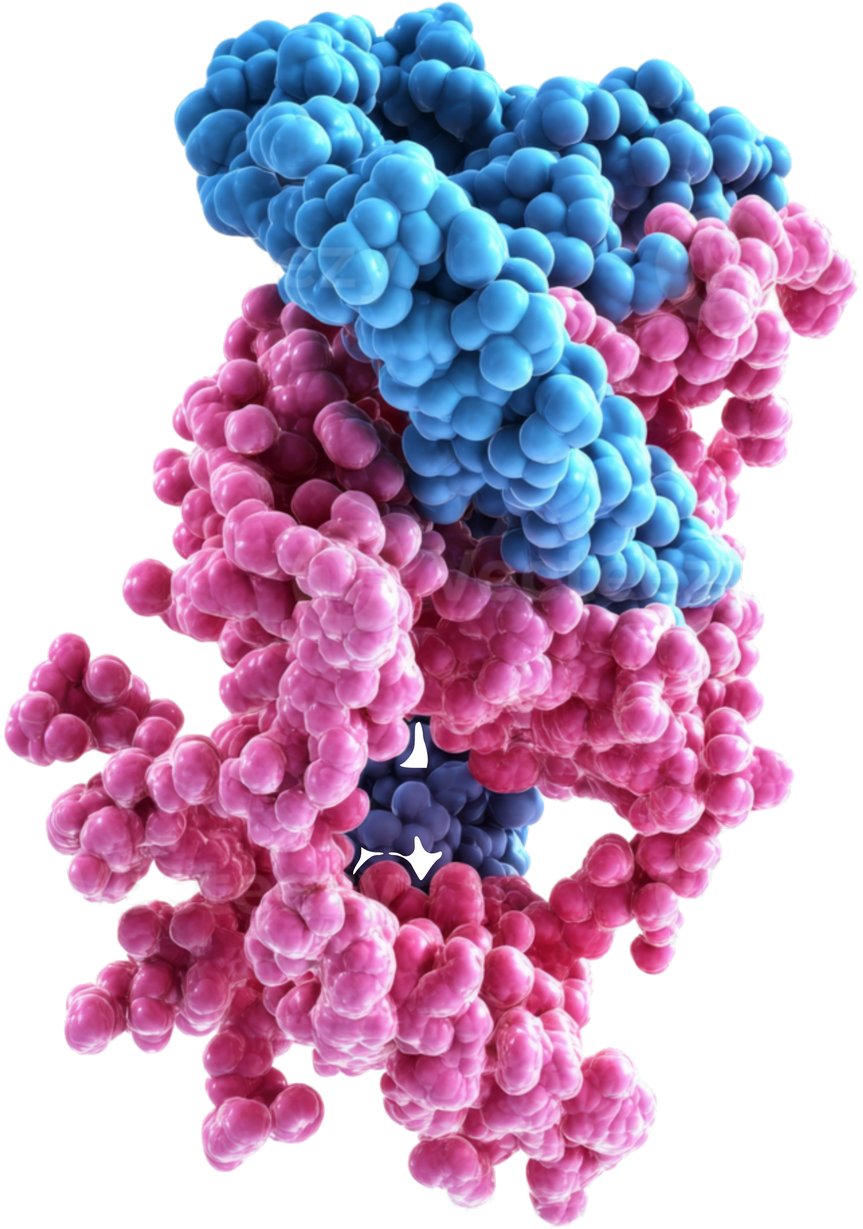
Golden age of induced proximity (2015–present)

Exponential growth in induced proximity publications

Ray Deshaies



Schreiber, S. L. *Cell* **2021**, *184* (1), 3–9.



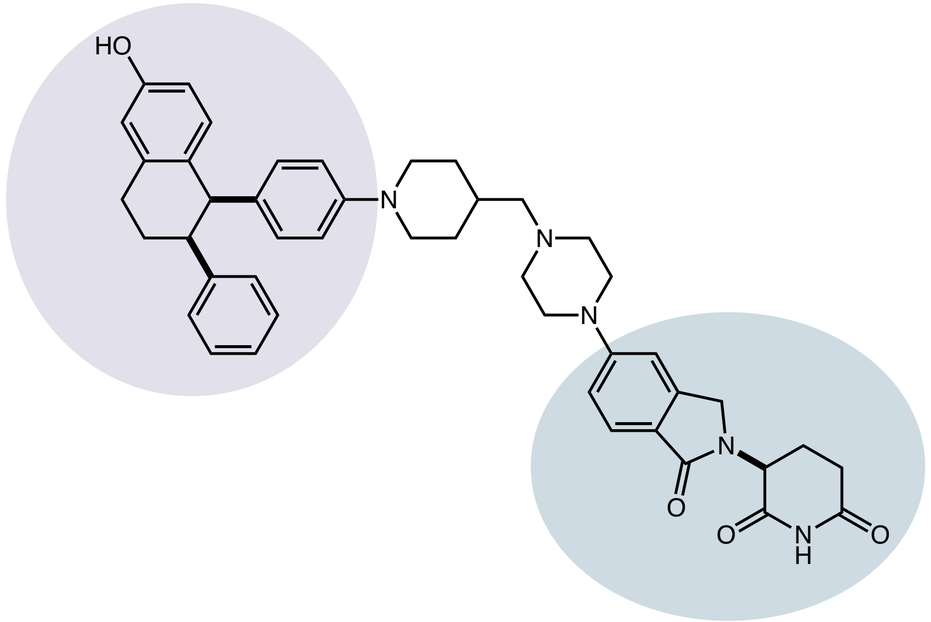
Modern Design of Chemical Inducers of Proximity

- *PROTACs and Bivalent Structures*
- *Molecular Glues*

Modern PROTACs defined

ARV-471

ERα

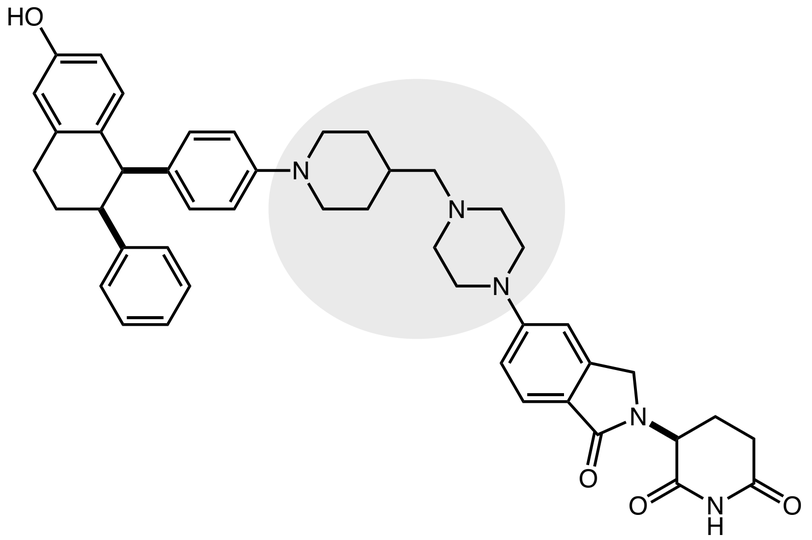


CRBN

PROTACs are generally **bivalent**: they contain an E3 ligase warhead and a protein of interest (POI) warhead

Modern PROTACs defined

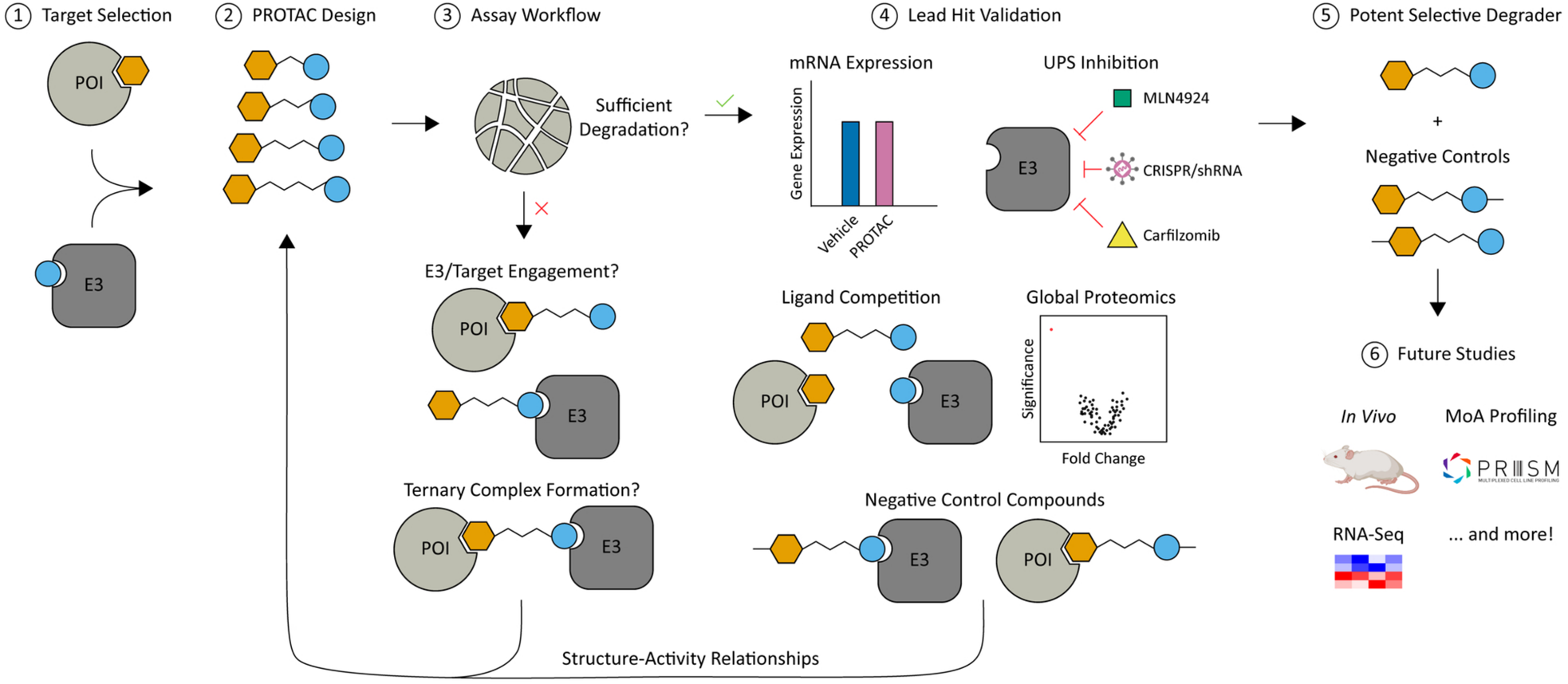
ARV-471



PROTACs are generally **bivalent**: they contain an E3 ligase warhead and a protein of interest (POI) warhead

The correct **linker** choice is essential for PROTAC function: **Flexible** or **Rigid**. **Alkyl, PEG, or Advanced**

Design of PROTACs

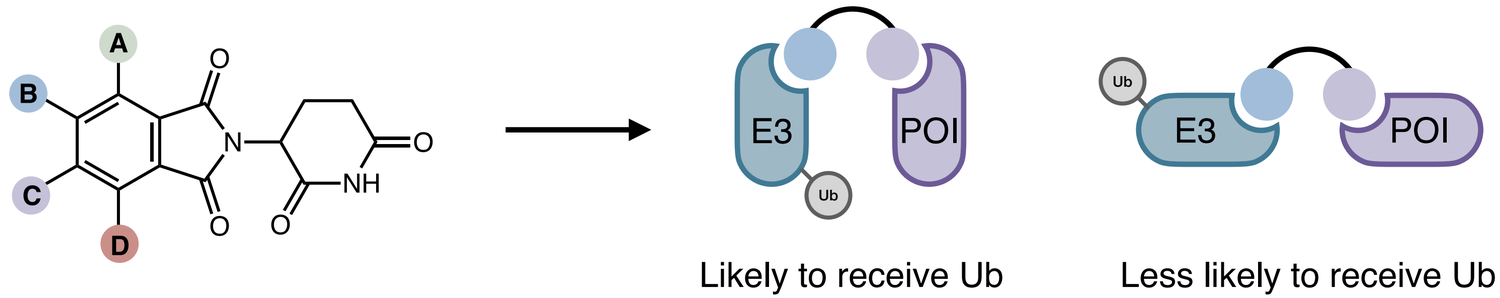


Tran, N. L.; Leconte, G. A.; Ferguson, F. M. *Curr Protoc* **2022**, *2* (12), e611.

Differences from design of traditional small molecules

Ligase–Target combination must be compatible

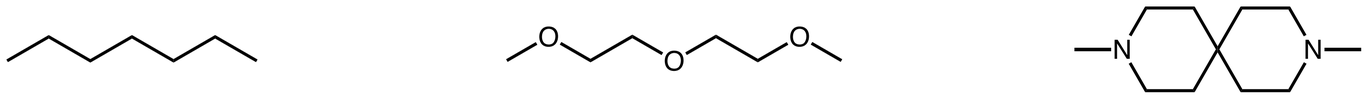
Exit vectors must be chosen for correct ternary complex formation



Likely to receive Ub

Less likely to receive Ub

Linker choice is critical

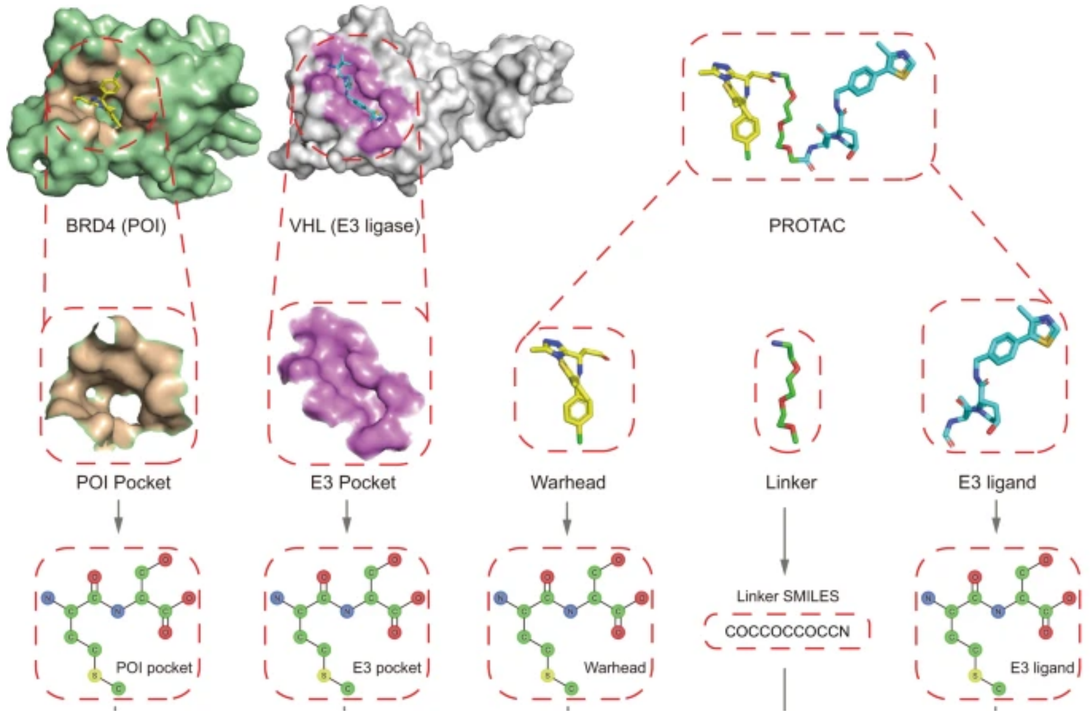


Alkyl

PEG

Advanced

AI in PROTAC design



Can train AI with compatible E3 ligase–target combinations

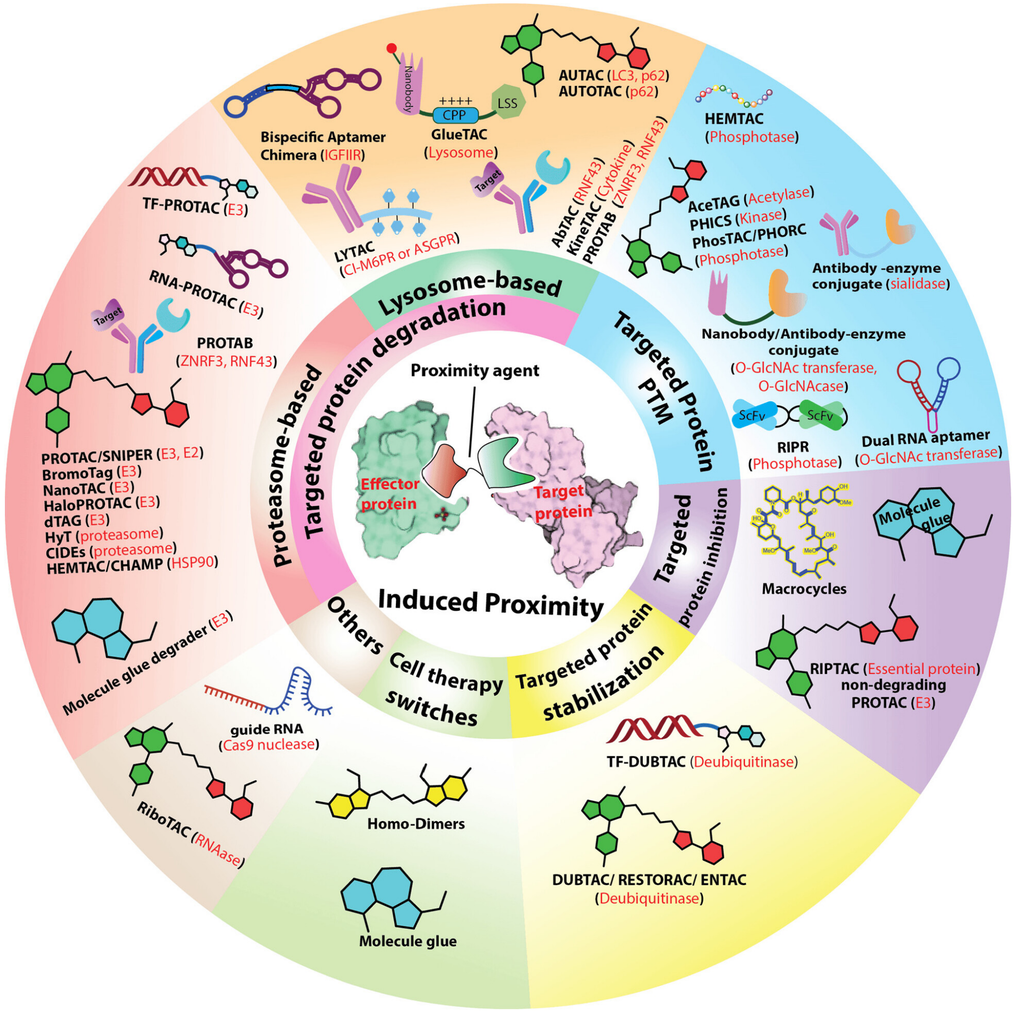
Fischer lab

Database of > 200 global proteomics of degrader responses from various molecules

Li, F.; Hu, Q.; Zhang, X.; Sun, R.; Liu, Z.; Wu, S.; Tian, S.; Ma, X.; Dai, Z.; Yang, X.; Gao, S.; Bai, F. *Nat Commun* **2022**, *13* (1), 7133.

Tran, N. L.; Leconte, G. A.; Ferguson, F. M. *Curr Protoc* **2022**, *2* (12), e611.

Other types of bifunctional modalities



Liu, X.; Ciulli, A. *ACS Cent. Sci.* **2023**, *9* (7), 1269–1284.

Winter, G. E. *ACS Chem. Biol.* **2024**, *19* (10), 2089–2102.

Other bivalent small molecule modalities: DUBTACs

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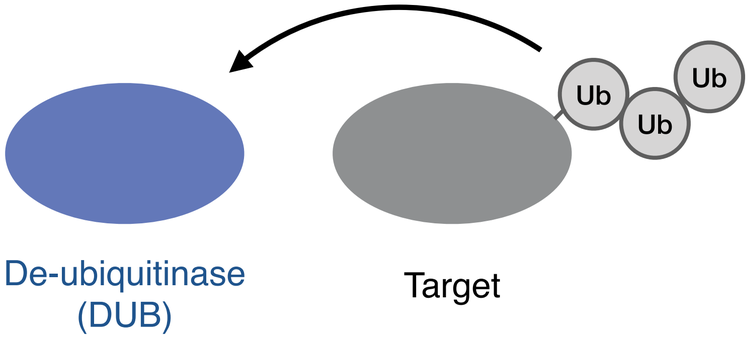
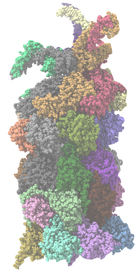
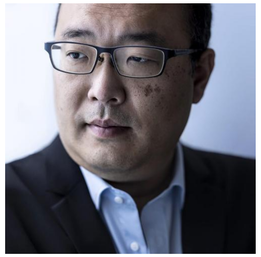
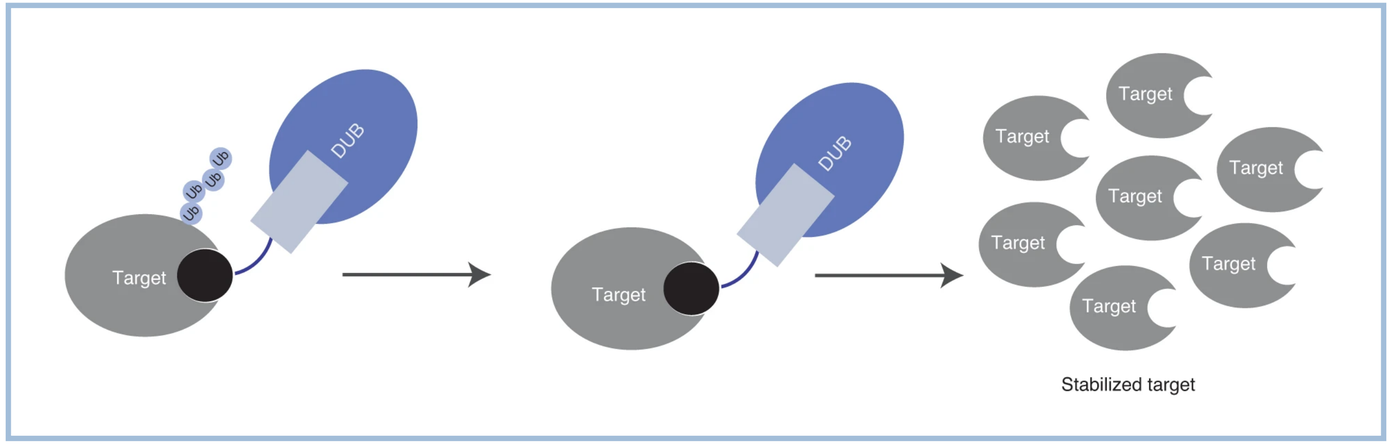




Not degraded by
26S proteasome



Nomura Lab



Induced proximity of DUBs can stabilize a given target protein

VICINITAS THERAPEUTICS

Spun out into company

Henning, N. J.; Boike, L.; Spradlin, J. N.; Ward, C. C.; Liu, G.; Zhang, E.; Belcher, B. P.; Brittain, S. M.; Hesse, M. J.; Dovala, D.; McGregor, L. M.; Valdez Misiolek, R.; Plasschaert, L. W.; Rowlands, D. J.; Wang, F.; Frank, A. O.; Fuller, D.; Estes, A. R.; Randal, K. L.; Panidapu, A.; McKenna, J. M.; Tallarico, J. A.; Schirle, M.; Nomura, D. K. *Nat Chem Biol* **2022**, *18* (4), 412–421.

Other bivalent small molecule modalities: Targeted protein re-localization

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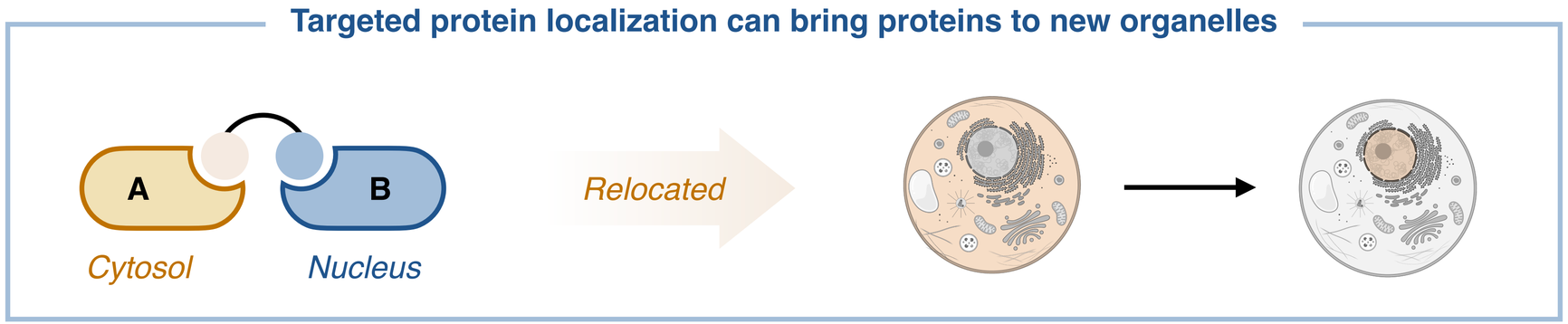


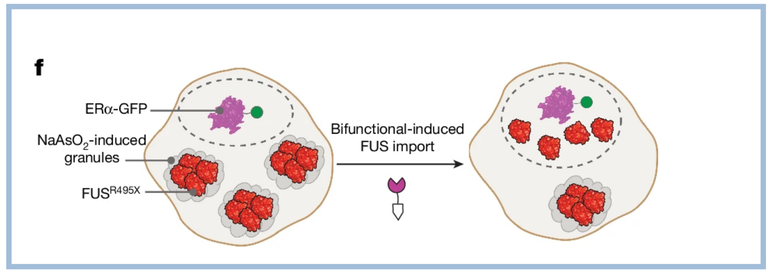
Gibson, W. J.; Sadagopan, A.; Shoba, V. M.; Choudhary, A.; Meyerson, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **2023**, *145* (48), 26028–26037.

Ng, C. S. C.; Liu, A.; Cui, B.; Banik, S. M. *Nature* **2024**, *633* (8031), 941–951.

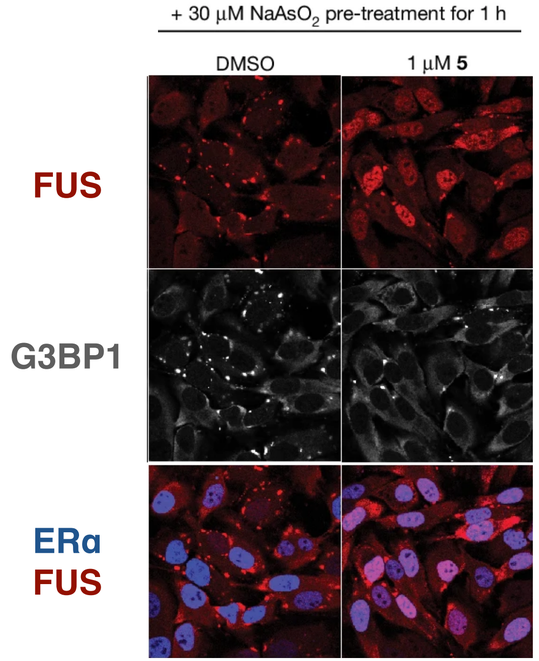
Other bivalent small molecule modalities: Targeted protein re-localization

Targeted protein localization can bring proteins to new organelles

Steven Banik Lab



Can relocate proteins outside of stress granules!



Fewer granules upon ligand treatment

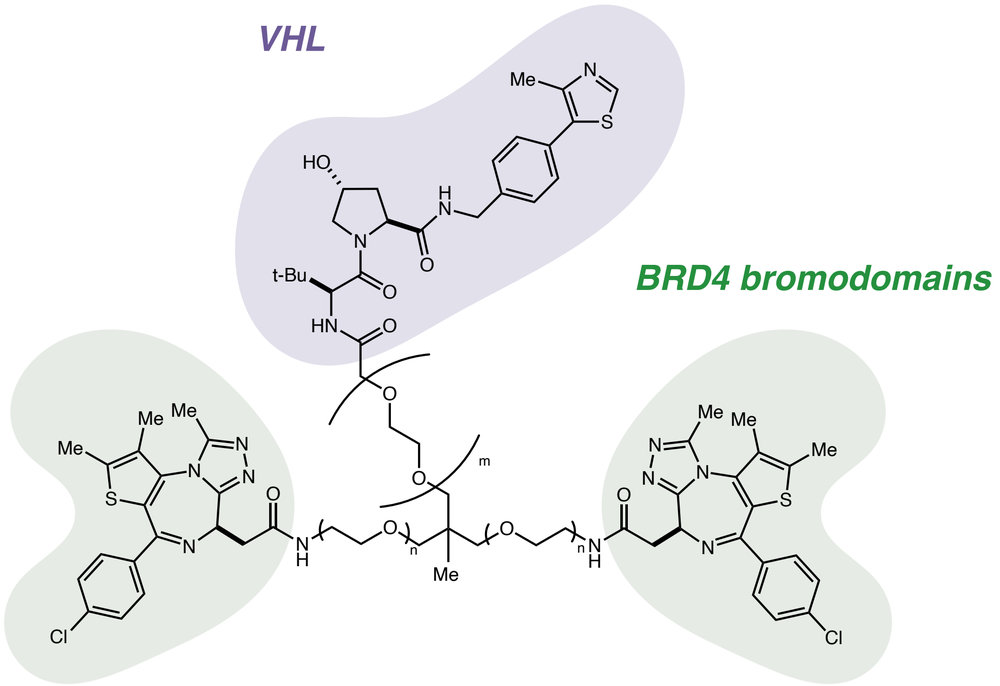
Gibson, W. J.; Sadagopan, A.; Shoba, V. M.; Choudhary, A.; Meyerson, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **2023**, *145* (48), 26028–26037.

Ng, C. S. C.; Liu, A.; Cui, B.; Banik, S. M. *Nature* **2024**, *633* (8031), 941–951.

Trivalent small molecules

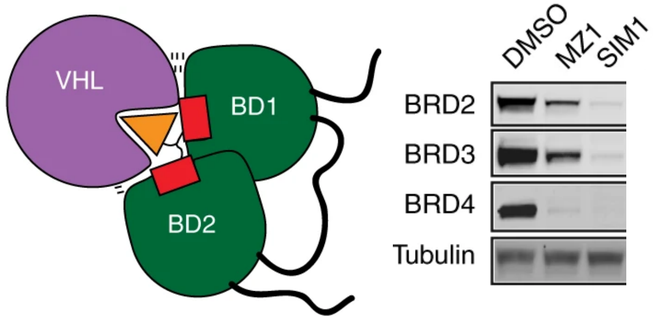
Trivalent small molecules

Ciullli Lab



SIM-1

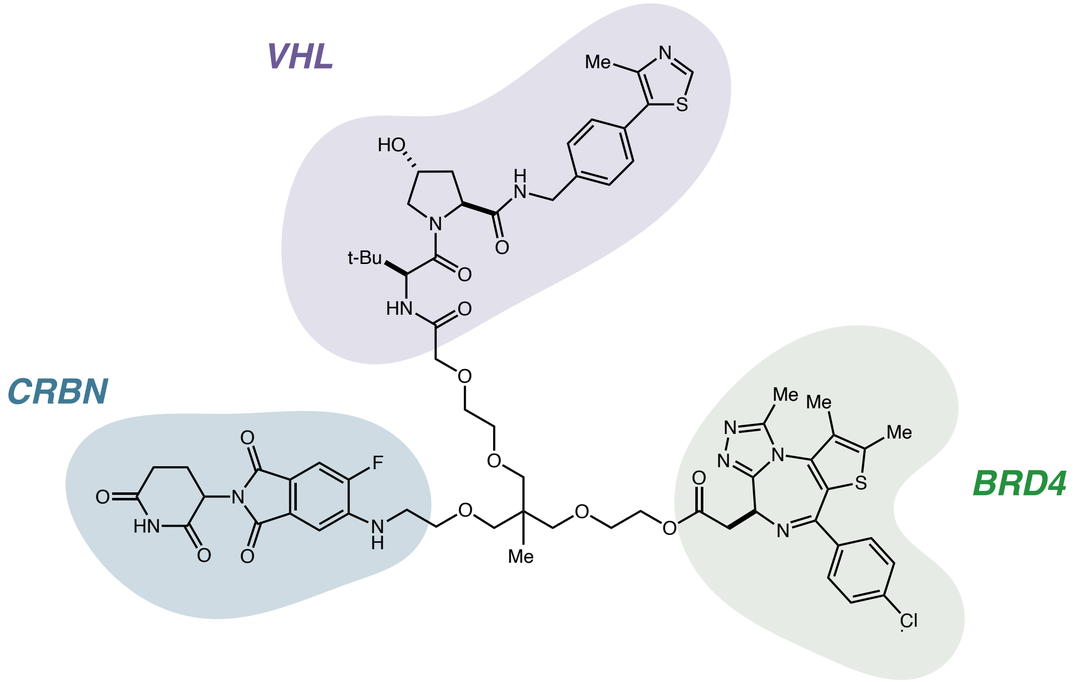
Better avidity = better degradation



Imaide, S.; Riching, K. M.; Makukhin, N.; Vetma, V.; Whitworth, C.; Hughes, S. J.; Trainor, N.; Mahan, S. D.; Murphy, N.; Cowan, A. D.; Chan, K.-H.; Craigon, C.; Testa, A.; Maniaci, C.; Urh, M.; Daniels, D. L.; Ciulli, A. *Nat Chem Biol* **2021**, *17* (11), 1157–1167.

Trivalent small molecules

Ciullli Lab



AB-3067

Two ligases, 1 target
= more potent degradation

Dual Ligase Recruitment
- Potent and rapid degradation ✓
- Additive ubiquitination and degradation from both ligases ✓
- Minimal cross-ligase degradation ✓

BET Degradation

BRD4 $D_{max\ 50}$: 0.6 nM; λ_{max}: 2.7 h^{-1}
BRD3 $D_{max\ 50}$: 0.4 nM; λ_{max}: 3.3 h^{-1}
BRD2 $D_{max\ 50}$: 2.0 nM; λ_{max}: 2.4 h^{-1}

Bond, A. G.; Muñoz i Ordoño, M.; Bisbach, C. M.; Craigon, C.; Makukhin, N.; Caine, E. A.; Nagala, M.; Urh, M.; Winter, G. E.; Riching, K. M.; Ciulli, A. *J. Am. Chem. Soc.* **2024**, *146* (49), 33675–33711.

Biologics: LyTACs and PROTAbs

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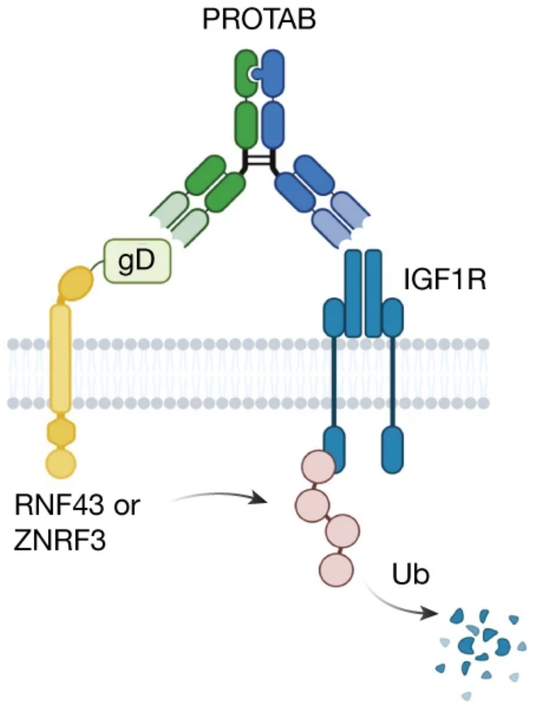

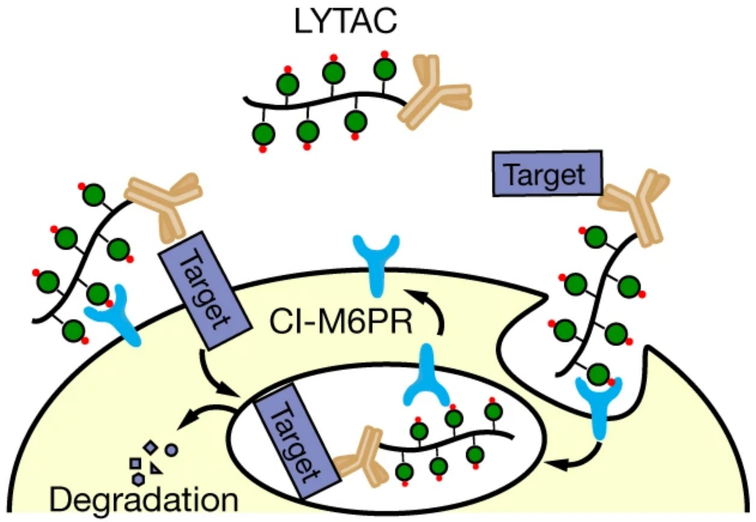

Allow for facile degradation of cell surface targets

Banik, S. M.; Pedram, K.; Wisnovsky, S.; Ahn, G.; Riley, N. M.; Bertozzi, C. R. Nature 2020, 584 (7820), 291–297.

Marei, H. et. al. *Nature* **2022**, *610* (7930), 182–189.

Biologics: Bispecific T-cell engagers (BiTEs)

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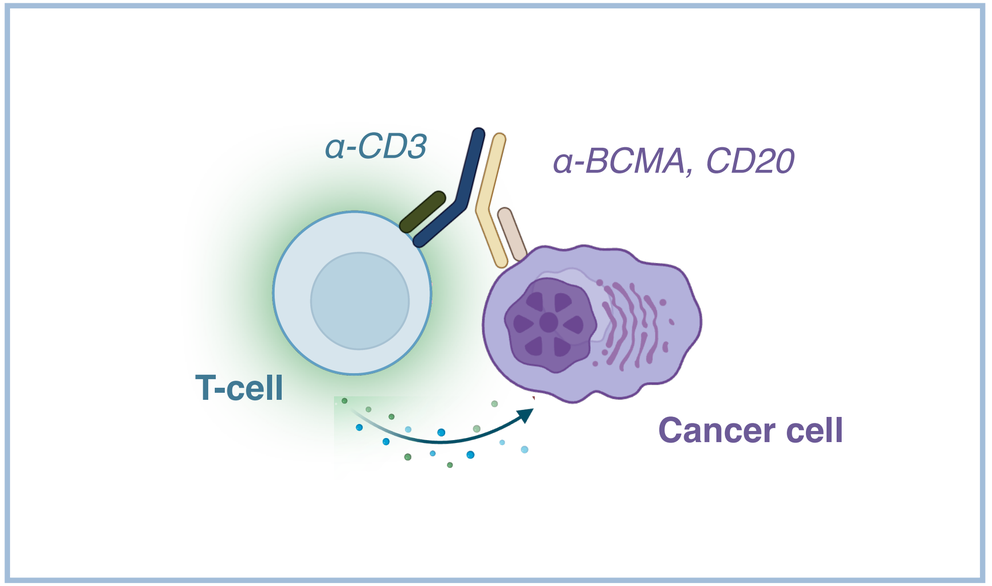
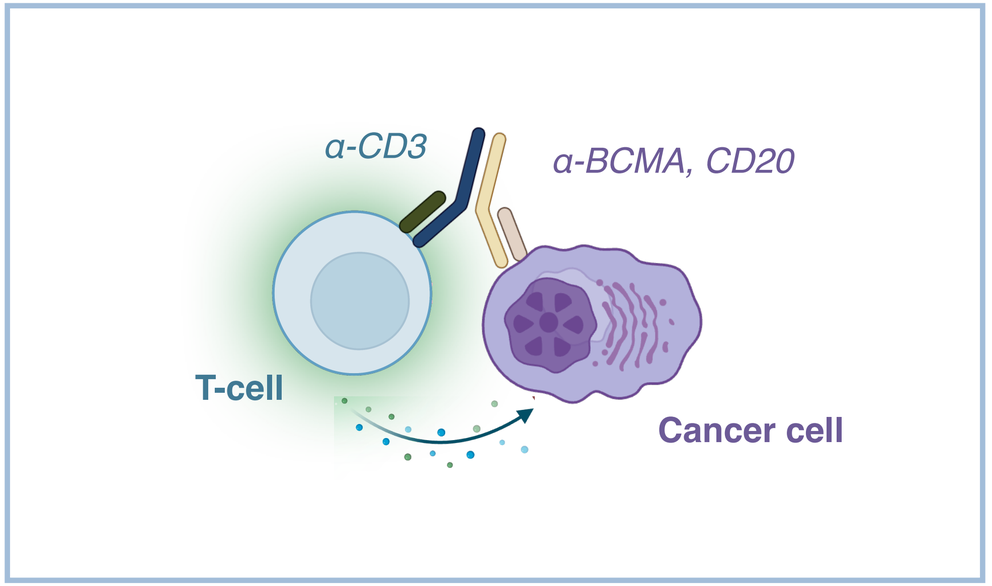


Tian, Z.; Liu, M.; Zhang, Y.; Wang, X. *Journal of Hematology & Oncology* **2021**, *14* (1), 75.

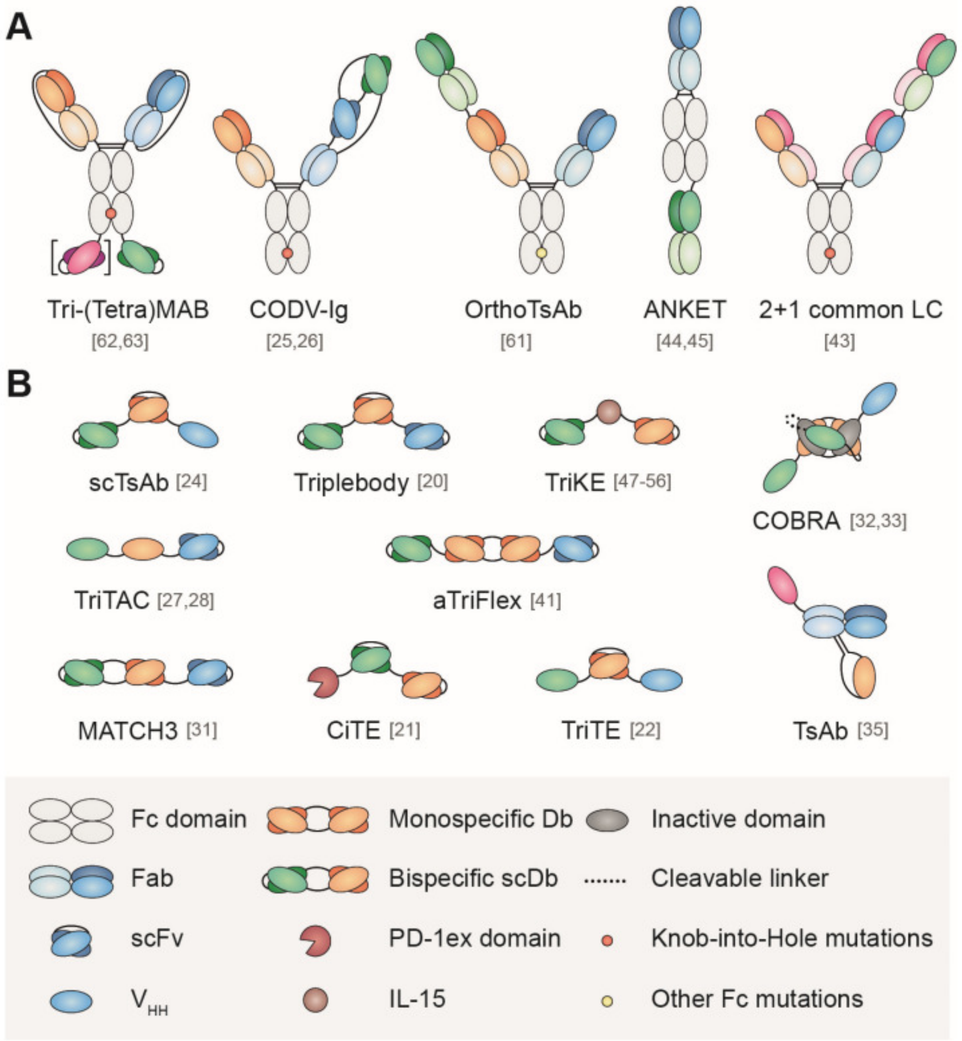
Biologics: Bispecific T-cell engagers (BiTEs)



5 BiTEs approved by FDA for use in myelomas and lymphomas

Tian, Z.; Liu, M.; Zhang, Y.; Wang, X. *Journal of Hematology & Oncology* **2021**, *14* (1), 75.

Biologics: Multispecific antibodies

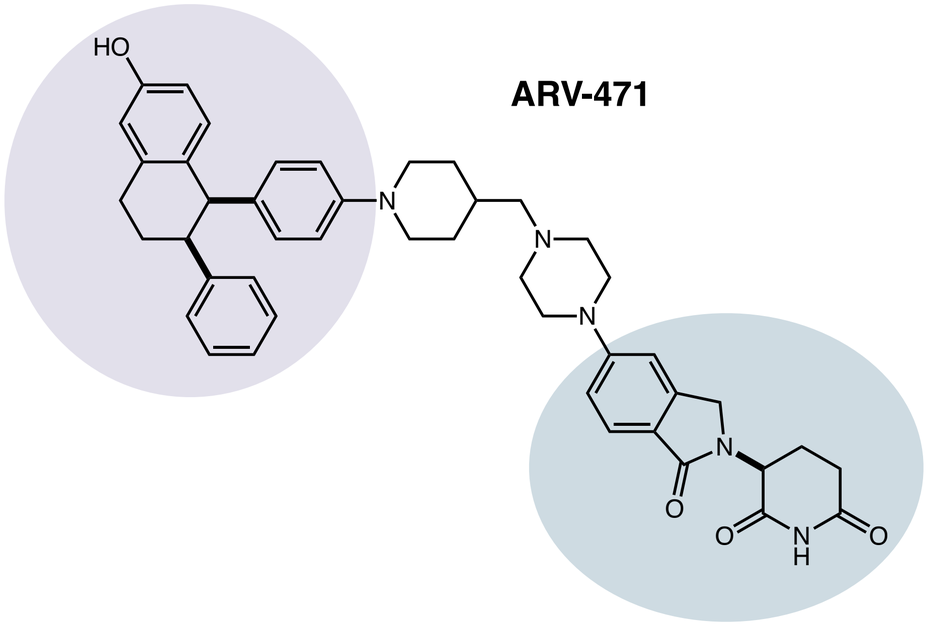


Tapia-Galisteo, A.; Compte, M.; Álvarez-Vallina, L.; Sanz, L. *Theranostics* **2023**, *13* (3), 1028–1041.

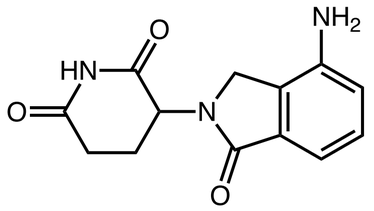
Molecular glues defined

Molecular glues defined

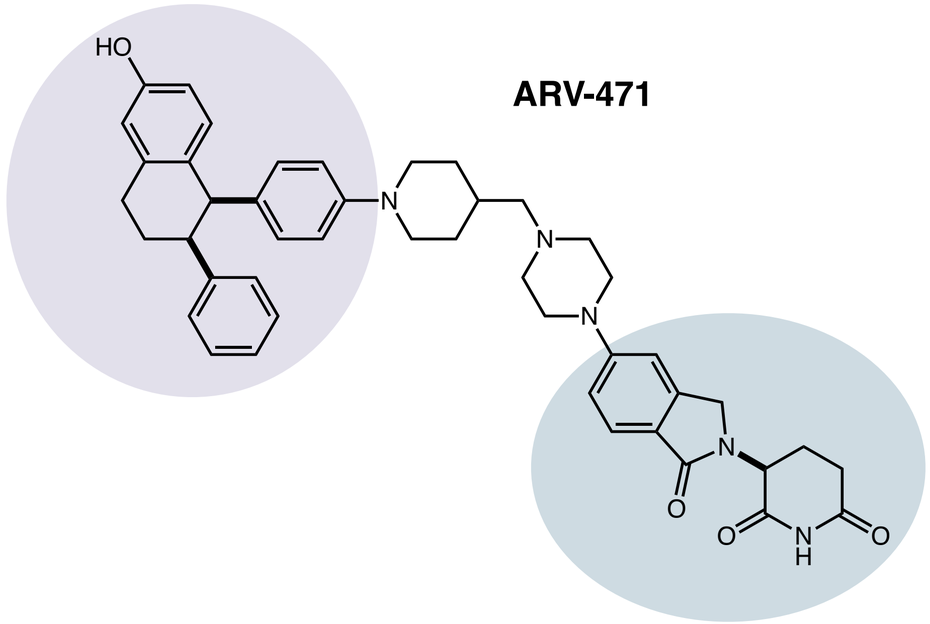
ARV-471



Lenalidomide



Molecular glues defined



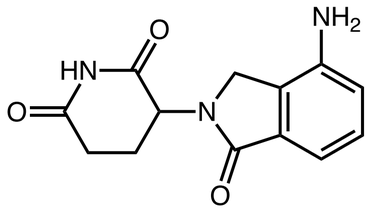
ARV-471

Bivalent molecule: Multiple warheads

Binding: Generally stepwise (although not always the case)

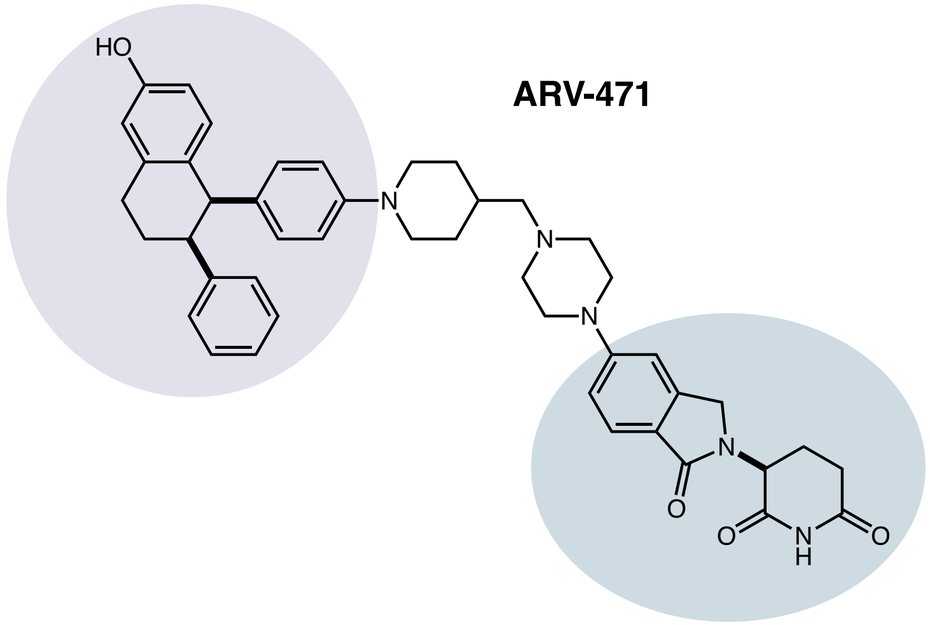
Hook Effect: Monovalent warheads saturate binding pockets at high concentrations

Lenalidomide



Molecular glues defined

ARV-471



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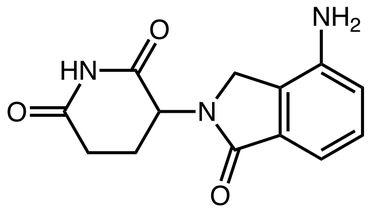
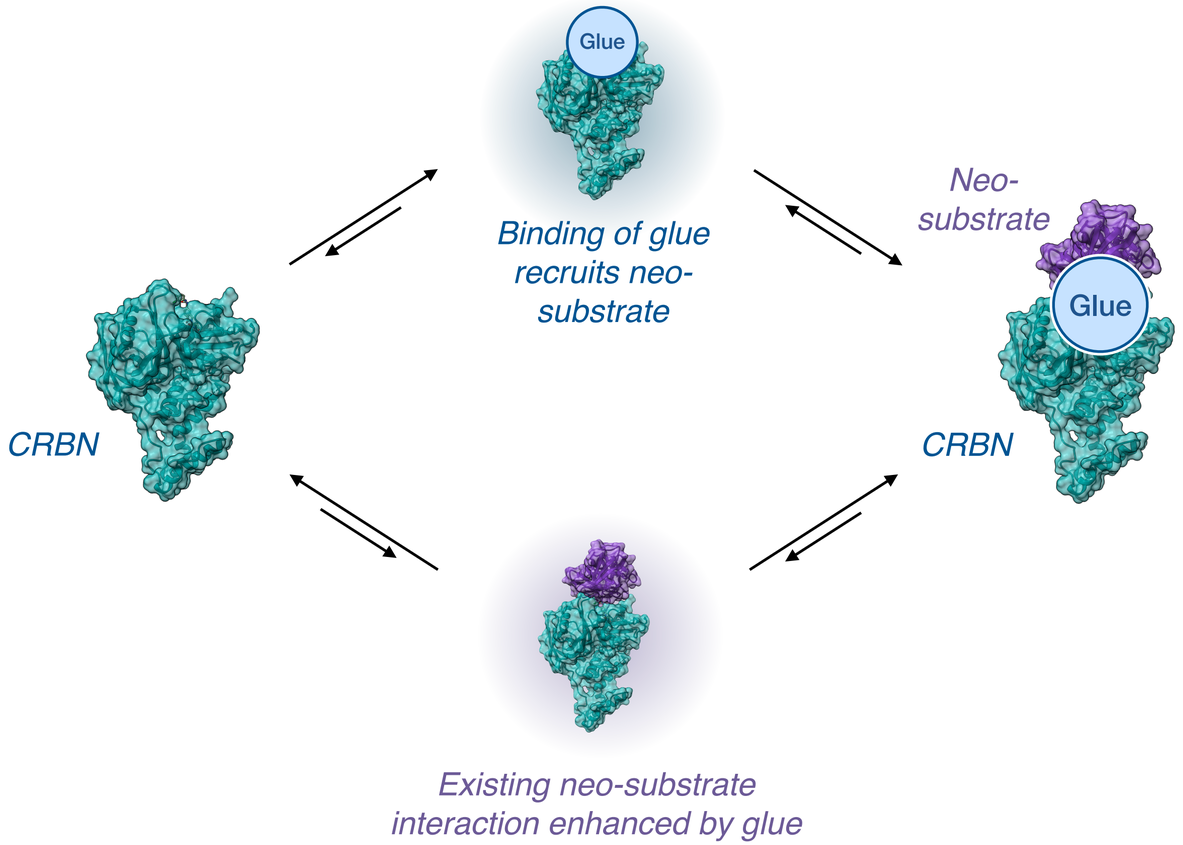


Monovalent molecule: Singular fragment

Binding: Usually concerted, but can be stepwise, or both!

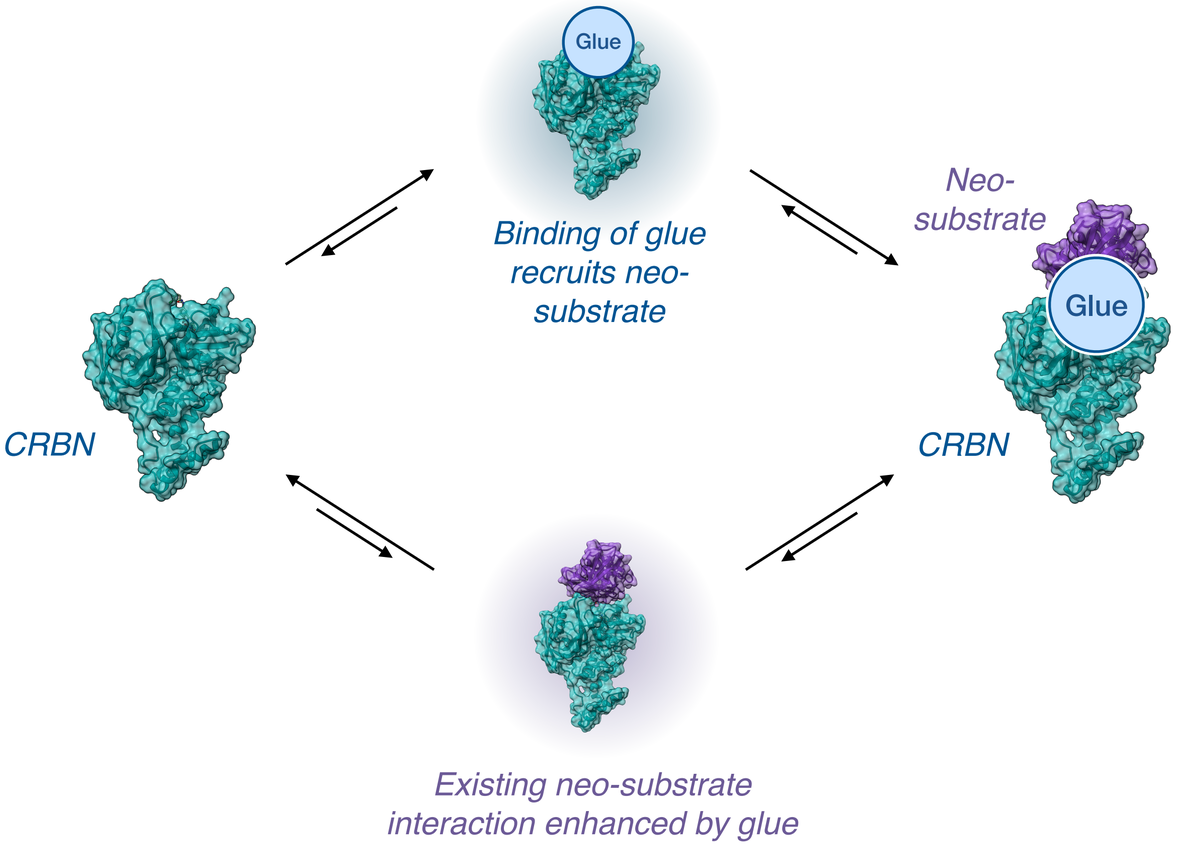
Hook Effect: Generally not seen, as the interface between targeted proteins is critical for binding

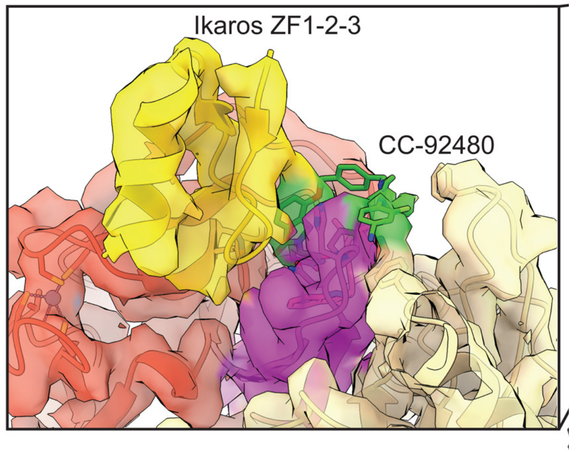
Binding modes of molecular glues



Rui, H.; Ashton, K. S.; Min, J.; Wang, C.; Potts, P. R. *RSC Chem. Biol.* **2023**, *4* (3), 192–215.

Binding modes of molecular glues





Glues create a binding surface between CBRN and Ikaros

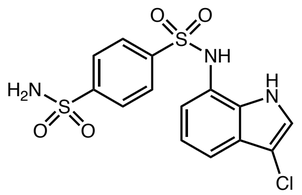
Watson, E. R.; Novick, S.; Matyskiela, M. E.; Chamberlain, P. P.; H. de la Peña, A.; Zhu, J.; Tran, E.; Griffin, P. R.; Wertz, I. E.; Lander, G. C. *Science* **2022**, *378* (6619), 549–553.

Rui, H.; Ashton, K. S.; Min, J.; Wang, C.; Potts, P. R. *RSC Chem. Biol.* **2023**, *4* (3), 192–215.

How are molecular glues commonly designed?

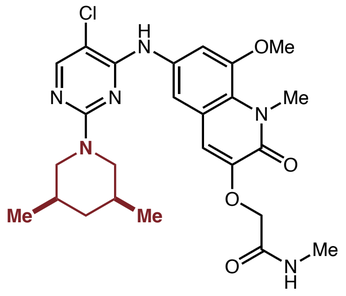
Discovery of glues by serendipity

Discovery of glues by serendipity



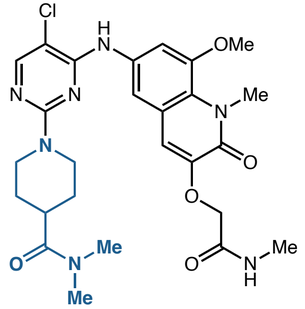
Indisulam

Solid tumor treatment



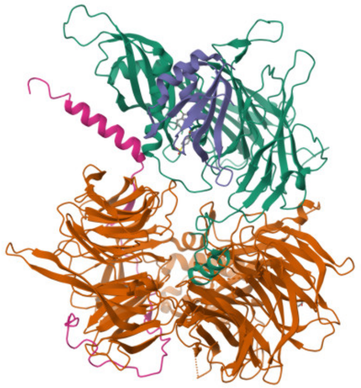
BI-3802

BCL6 Degrader

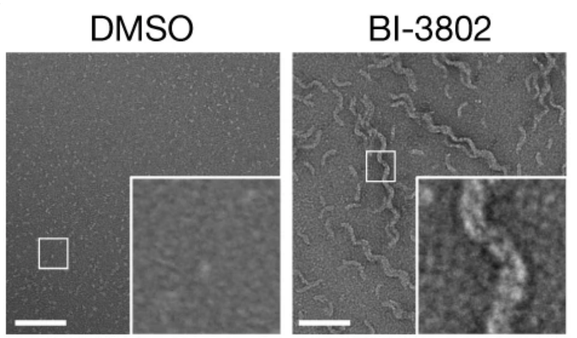


BI-3812

BCL6 Inhibitor



Indisulam found to induce ternary complex with DCAF15 and RBM39



BI-3082 induces BCL6 polymers before degradation

Han, T.; Goralski, M.; Gaskill, N.; Capota, E.; Kim, J.; Ting, T. C.; Xie, Y.; Williams, N. S.; Nijhawan, D. *Science* **2017**, *356* (6336), eaal3755.

Słabicki, M. et. al. *Nature* **2020**, *588* (7836), 164–168.

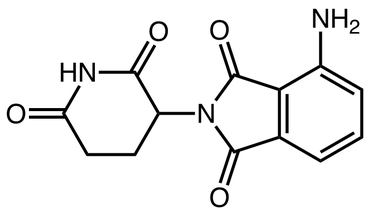
Modifying existing glues to serve new purposes

Modifying existing glues to serve new purposes

NOVARTIS

Looking for treatments for sickle cell disease

Pomalidomide



*Shown preclinically to increase levels of **γ-globin***

Globin gene cluster

Silenced upon birth

Fully functional in sickle cell patients

Mutated in sickle cell patients

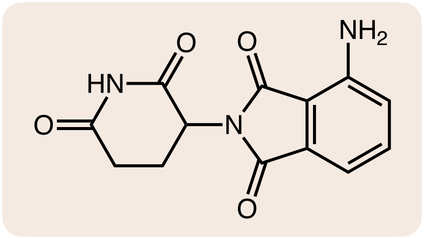
Ting, P. Y. et. al. *Science* **2024**, *385* (6704), 91–99.

Modifying existing glues to serve new purposes

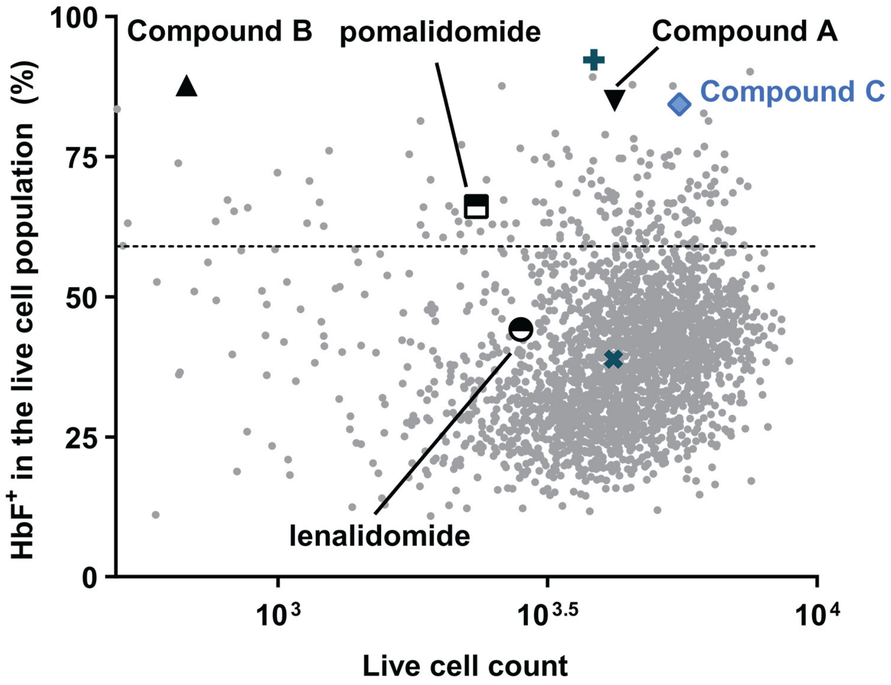
Pamela Ting

Jay Bradner

Screen library of ~3,000 CRBN binders



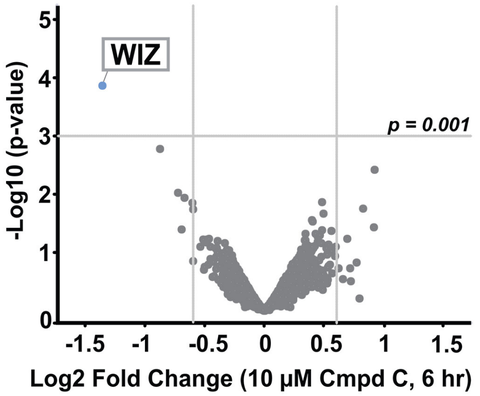
γ-Globin (HbF) production



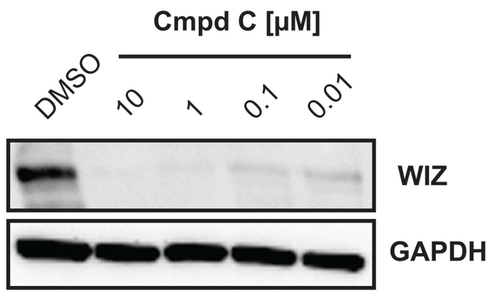
Pomalidomide derivatives lead to significant increases in HbF production!

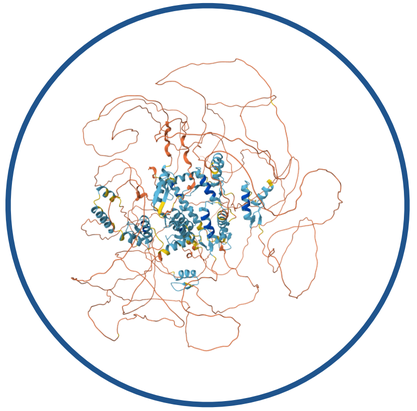
Ting, P. Y. et. al. *Science* **2024**, *385* (6704), 91–99.

Modifying existing glues to serve new purposes

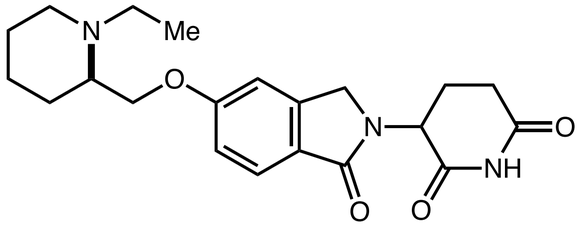


Selective over IKZF1 and IKZF3

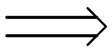


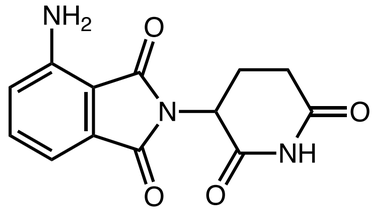


Transcription factor **WIZ** is the target of new glues



dWIZ-2 (after optimization)





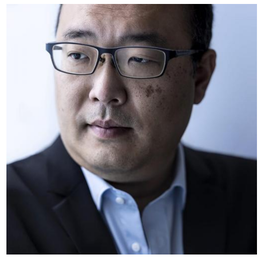
Pomalidomide

Small changes in exit vectors can completely alter substrate specificity

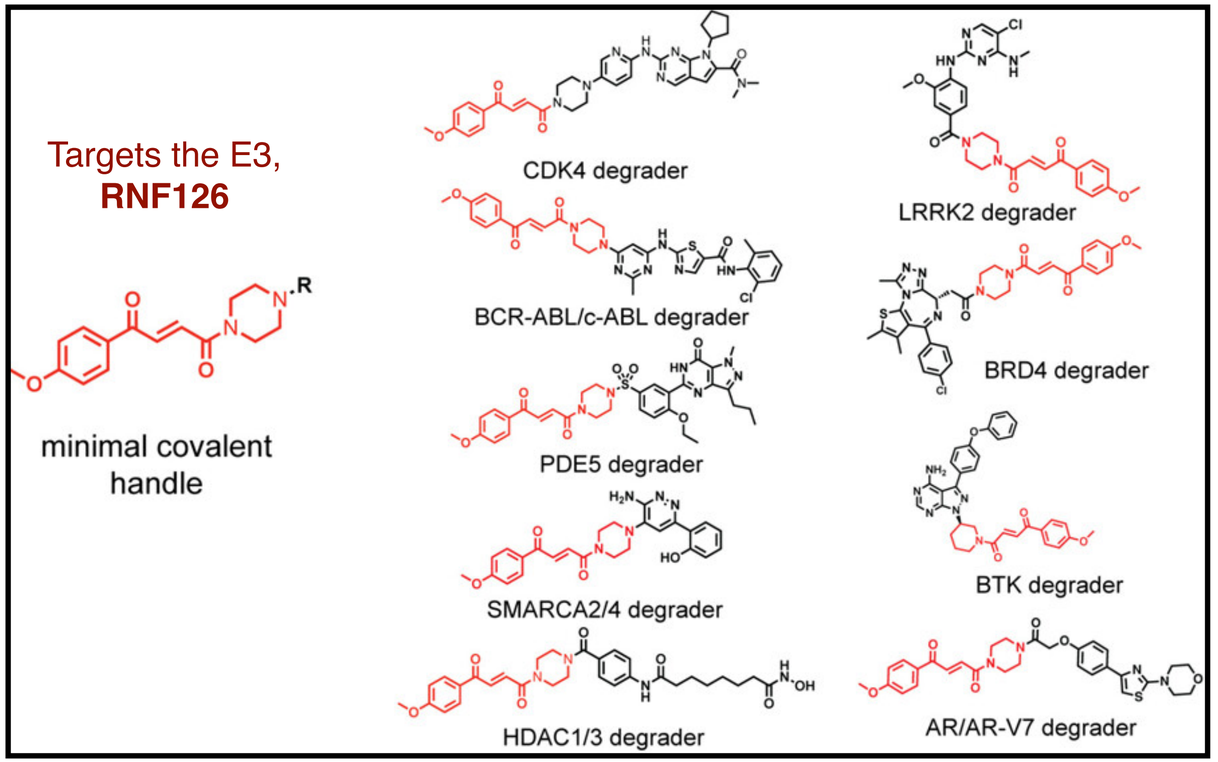
Ting, P. Y. et. al. *Science* **2024**, *385* (6704), 91–99.

Covalent glues

Covalent glues



Nomura Lab



Are these technically "glues"?

*Regardless of definition, this greatly reduces the **molecular weight** required for degradation*

Toriki, E. S.; Papatzimas, J. W.; Nishikawa, K.; Dovala, D.; Frank, A. O.; Hesse, M. J.; Dankova, D.; Song, J.-G.; Bruce-Smythe, M.; Struble, H.; Garcia, F. J.; Brittain, S. M.; Kile, A. C.; McGregor, L. M.; McKenna, J. M.; Tallarico, J. A.; Schirle, M.; Nomura, D. K. *ACS Cent. Sci.* **2023**, *9* (5), 915–926.

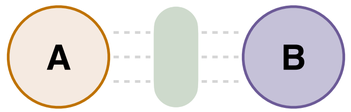
Outlook on future design

Outlook on future design

No truly “rational” method to design glues without preliminary knowledge / design!

> **“The truth is, you can only design when there’s already something known.”**
>
> *—Markus Warmuth, CEO of Monte Rosa*

Future of the field:



Glue any proteins you want

Far away from this goal, maybe AI will help

Molecular glues are beginning to stick. Chemical & Engineering News. https://cen.acs.org/pharmaceuticals/drug-discovery/Molecular-glues-startups-search-beyond-PROTACS/100/i29 (accessed 2025-02-07).

Clinical outlook of PROTACs and glues

PROTACs are currently targeting an array of diseases in clinical trials

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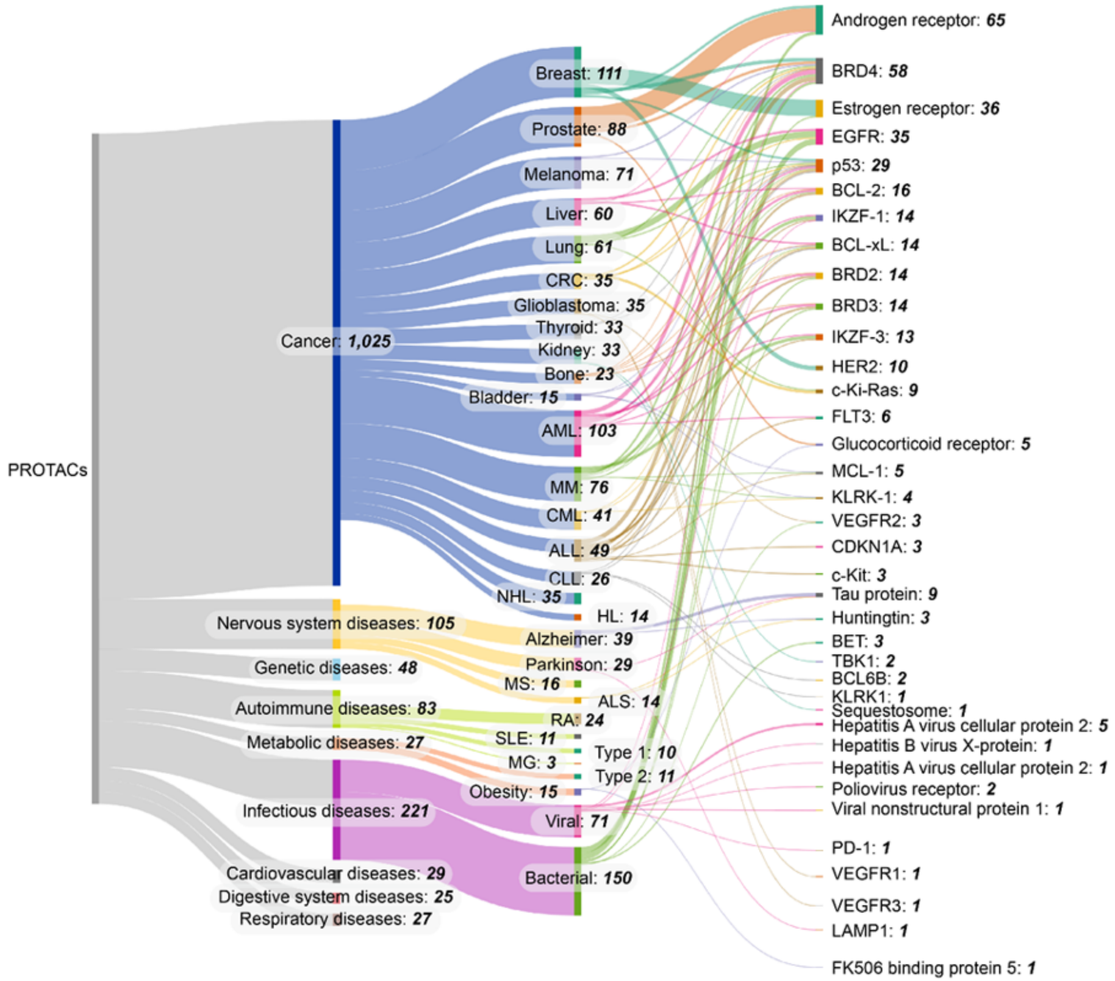


CRC: Colorectal cancer, AML: Acute myeloid leukemia, MM: Multiple myeloma, CML: Chronic myeloid leukemia, ALL: Acute lymphoblastic leukemia, CLL: Chronic lyphoblastic leukemia, NHL: Non-Hodgkins lyphoma, HL: Hodgkins lyphoma, MS: Multiple sclerosis, ALS: Amyotrophic lateral sclerosis, RA: Rheumatoid arthrtis, SLE: Systemic lupus erythematosus, Type 1: Type 1 diabetes, MG: Myastehia gravis, Type 2: Type 2 diabetes,

PROTACs are revolutionizing small molecule drug development. https://www.cas.org/resources/cas-insights/protac-drug-development (accessed 2025-02-07).

Clinical PROTAC startups have raised significant capital in recent years

Selection of TPD Companies

C4 Therapeutics

$182M IPO

nurix

$209M IPO

Monte Rosa THERAPEUTICS

$222M IPO

proxygen

$550M partnership with Merck

TRIANA Biomedicines

$110M Series A

DEGRON THERAPEUTICS

$22M Series A

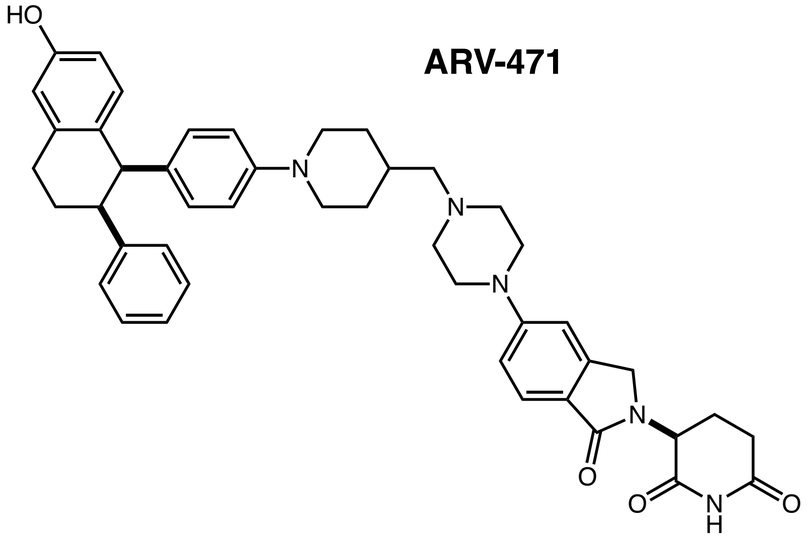
Targeted protein degradation is a powerful therapeutic strategy with over $3.5 billion in capital

Current PROTACs in Phase III

Craig Crews

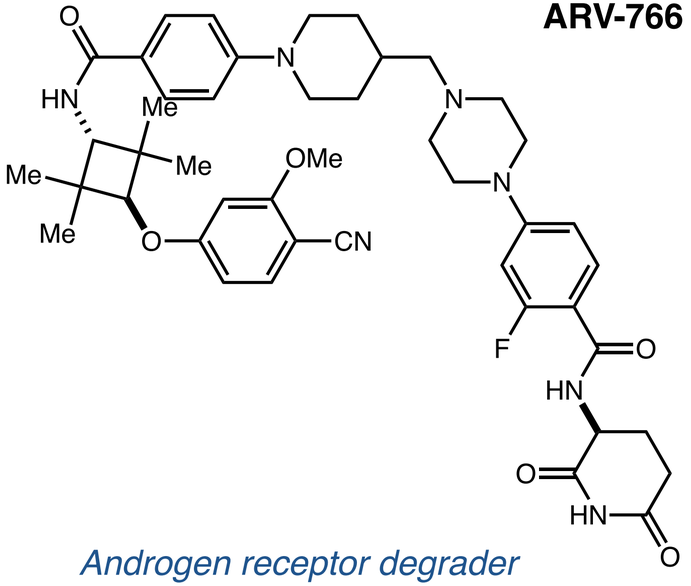
ARVINAS

ARV-471



Estrogen receptor degrader

ARV-766



Androgen receptor degrader

Other PROTACs in clinical trials

Treatment	Organization(s)	Target	Phase	Lead indication
Vepdegestrant (ARV-471)	Arvinas and Pfizer	Estrogen receptor	3	Metastatic breast cancer
ARV-766	Arvinas and Novartis	Androgen receptor	3	Metastatic castration-sensitive and castration-resistant prostate cancer
Bavdegalutamide (ARV-110)	Arvinas	Androgen receptor	1/2	Metastatic castration-resistant prostate cancer
ARV-102	Arvinas	LRRK2	1	Parkinson's disease
KT-474	Kymera Therapeutics	IRAK4	2	Hidradenitis suppurativa and atopic dermatitis
KT-333	Kymera Therapeutics	STAT3	1a/b	Refractory leukemias and lymphomas
NX-5948	Nurix Therapeutics	BTK	1a/b	B cell cancers
NX-2127	Nurix Therapeutics	BTK and IKZF	1b	B cell cancers

Arnold, C. *Nature Medicine* **2024**, *30* (11), 3030–3031.

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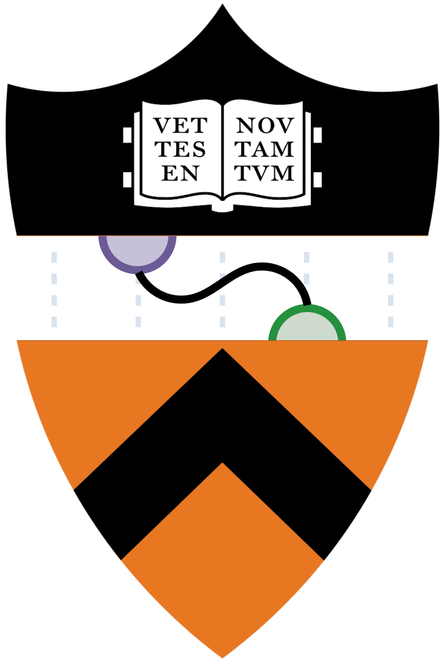
PROTACs can even enter the brain!

Arnold, C. *Nature Medicine* **2024**, *30* (11), 3030–3031.

Induced Proximity



Philip Raftopoulos

Group meeting: February 7th, 2025

Questions