PROteolysis TArgeting Chimera (PROTAC)
Targeted Intracellular Protein Degradation

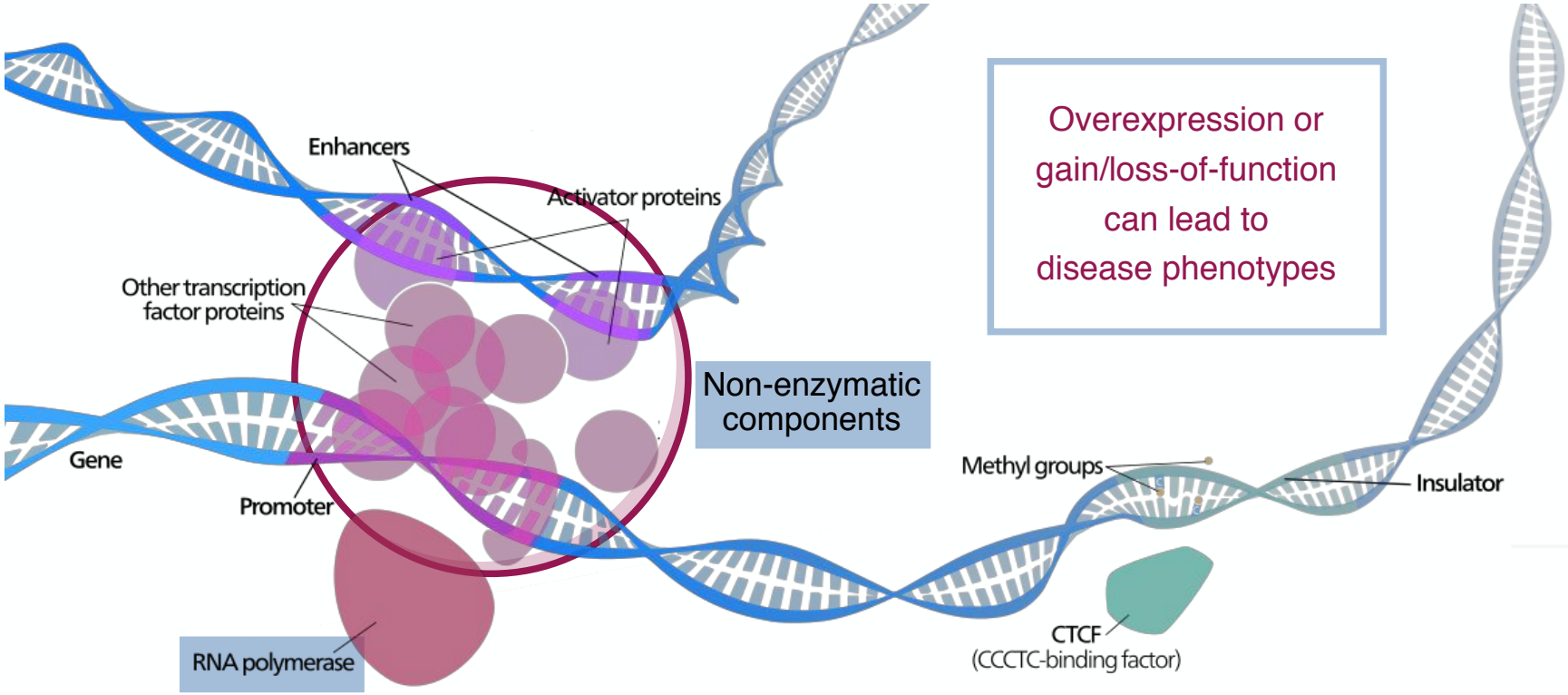
Literature presentation
Junyong Kim
May 23rd, 2019

Contemporary Drug Discovery

Small molecule inhibition has been a sccessful approach for drug discovery

Inhibition of drug target affects disease phenotype ⇨ Find high affinity inhibitor through SAR screening ⇨ Optimize phamacokinetics & pharmacodynamics ⇨ **In vivo test & clinical trial**

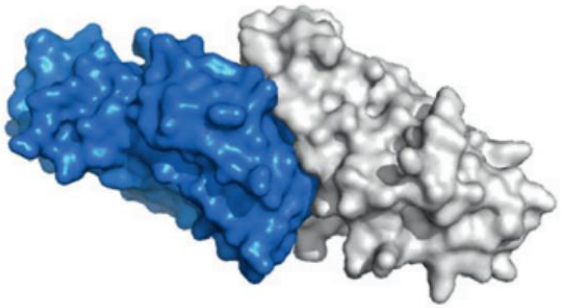
Only about 15% of the human proteome is '**druggable**'



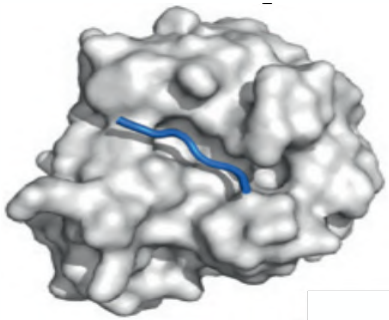
Russ, A.P.; Lampel, S. *Drug Discov. Today* **2005**, *10*, 1607–1610.

Druggable vs. Undruggable

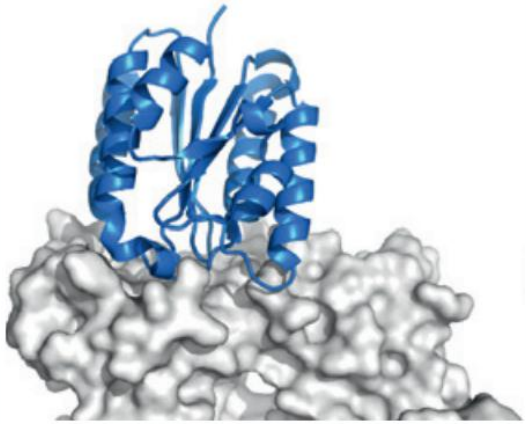
Protein–Protein interaction



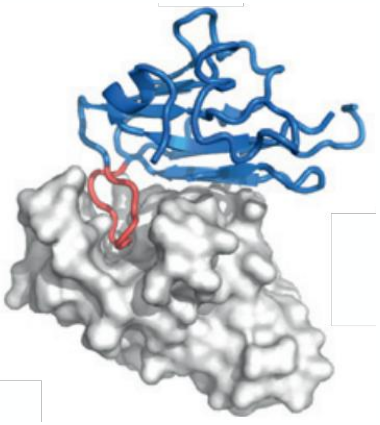
Protein–small molecule interaction



No dominant **Hot Segment**



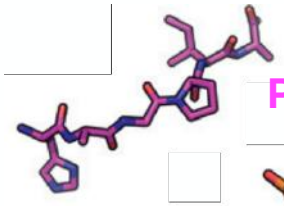
Dominant **Hot Segment**



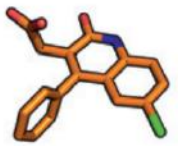
Surface area < 2500 Å^2

$K_d < 200$ nM

Amenable to small molecule inhibition



Peptides

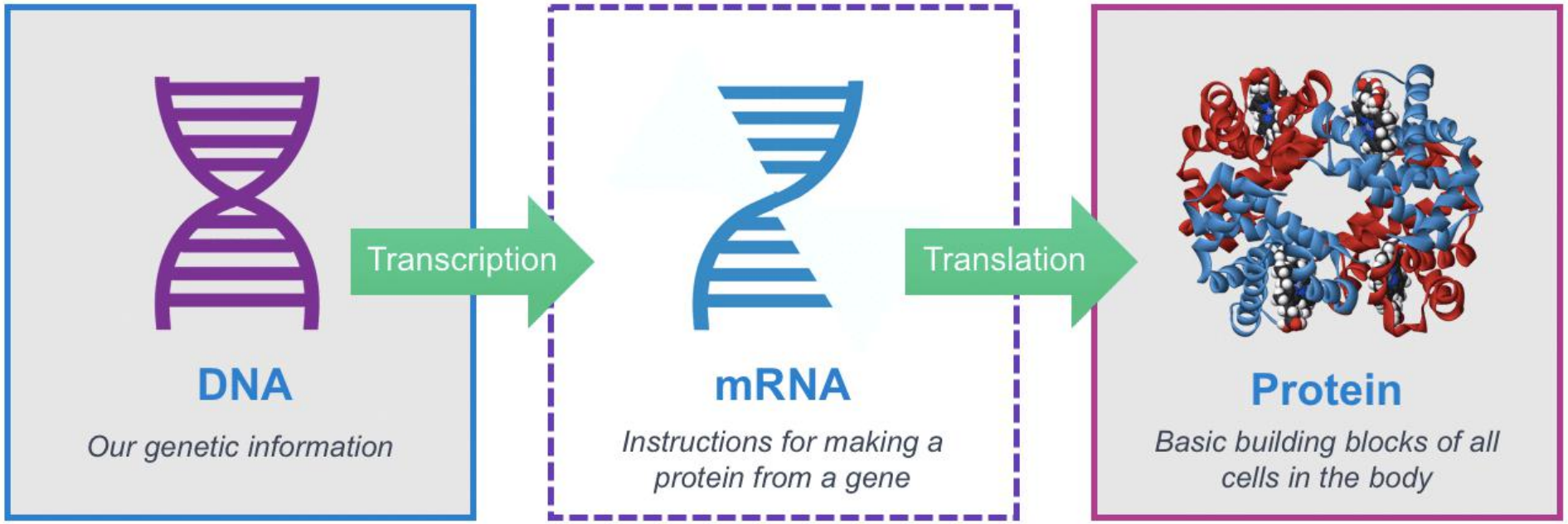


Small molecules

Biological approach is needed for undruggable targets

London, N.; Raveh, B.; Schueler-Furman, O. *Curr. Opin. Chem. Biol.* **2013**, *17*, 952–959.

Biological Approaches for Undruggable Targets



CRISPR-Cas 9

Fix defective genes by insertion or removal

First clinical trial started in 2016 (China), 2018 (EU)

RNAi

Prevent gene expression by inhibiting translation

First FDA approval (2018) Onpattro™ (amyloidosis)

Chemical genetics

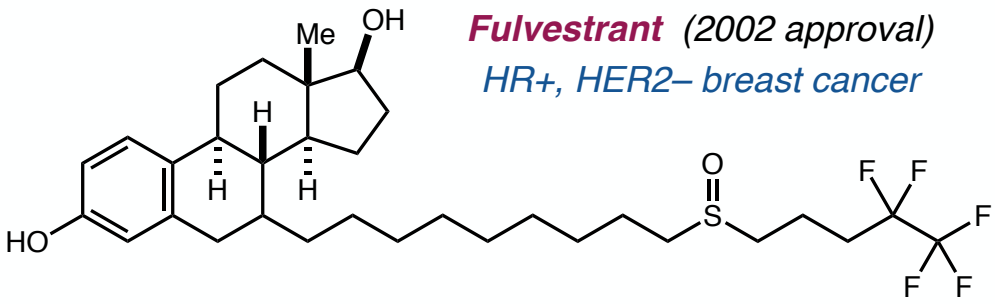
PROTAC

Degrade target proteins by ubiquitin–proteosome system

First clinical trial (2018) ARV-110 (prostate cancer)

Guo, J.; Liu, J.; Wei, W. *Cell Res.* **2019**, *29*, 179–180.

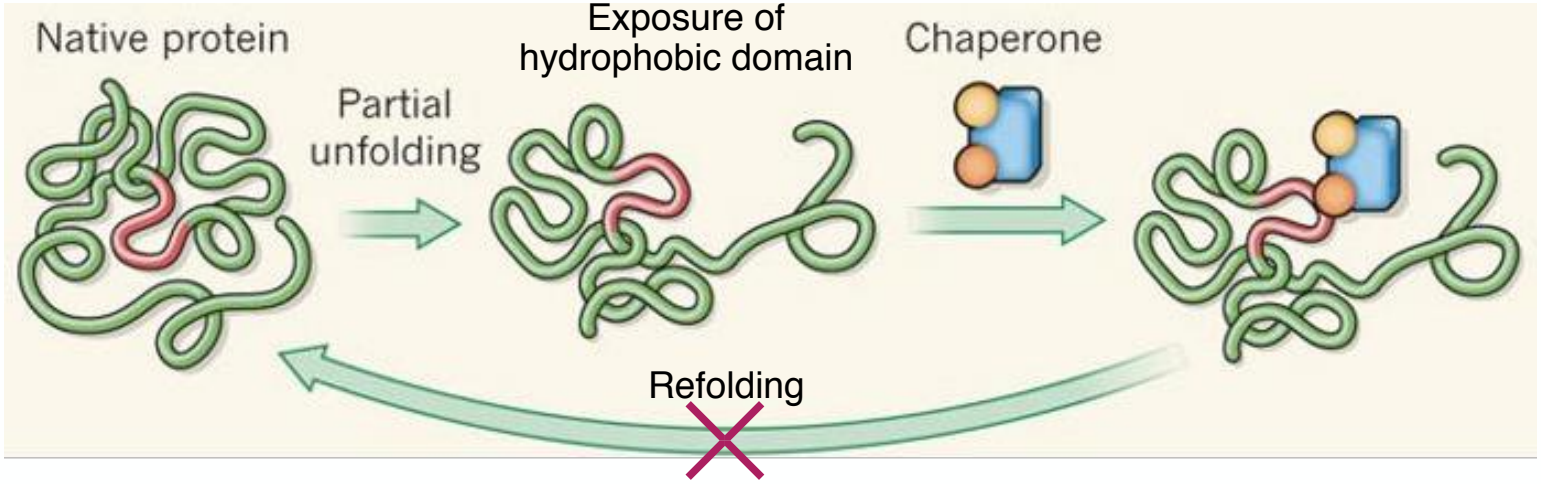
Selective Estrogen Receptor Downregulator (SERD)



Fulvestrant *(2002 approval)*

HR+, HER2– breast cancer

Decrease intracellular estrogen receptor (ERα) levels



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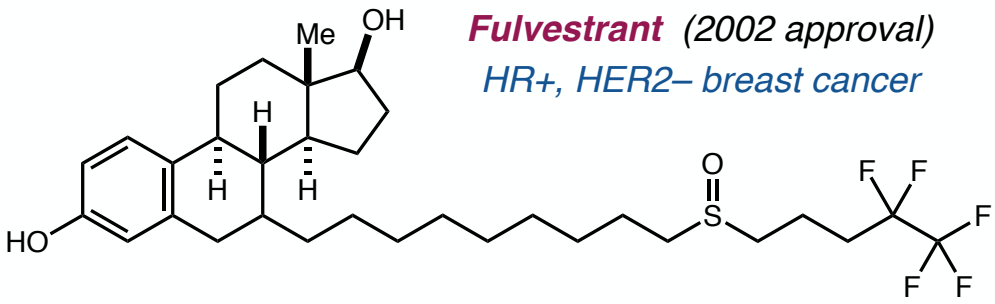

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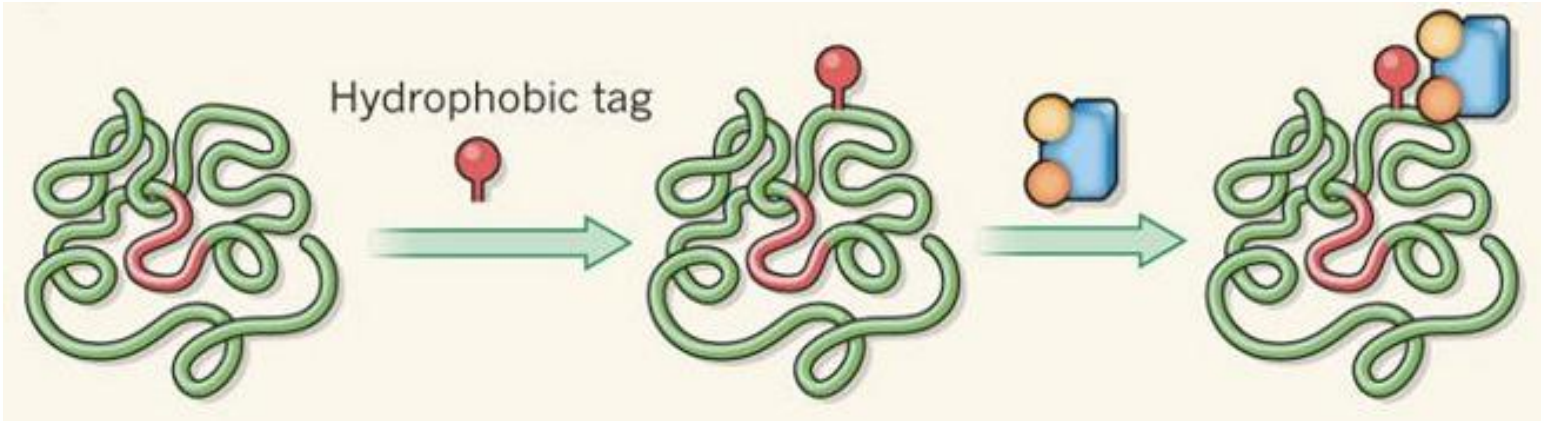
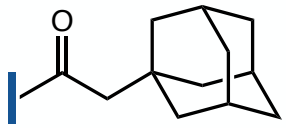


Protein degradation

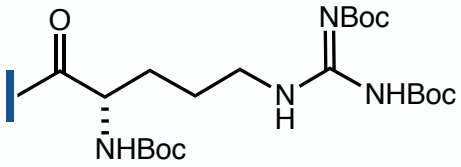
Hydrophobic tag to mimic misfolded protein

Hydrophobic Tagging (HyT)

Hydrophobic tags



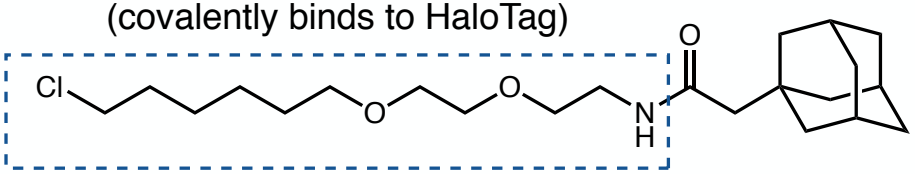
Adamantyl

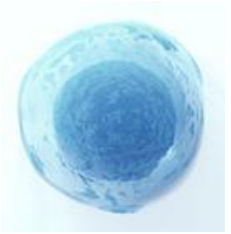


$(Boc)_3Arg$

Covalently attaching hydrophobic tags will induce protein degradation

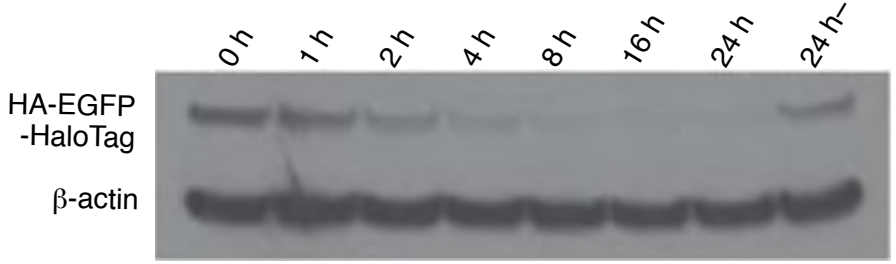
HaloTag reactive linker
(covalently binds to HaloTag)



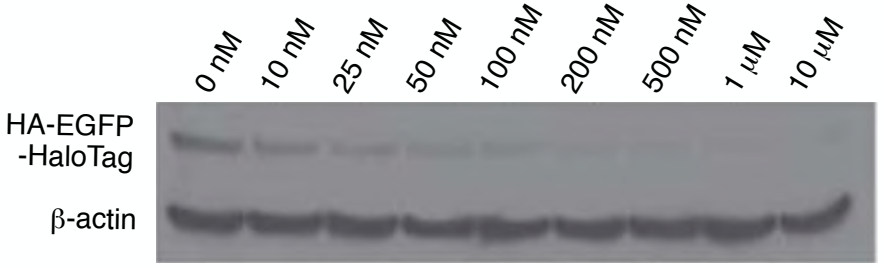


Cells expressing
EGFP–HaloTag

Time-dependent protein degradation

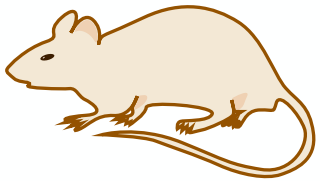


Dose-dependent protein degradation



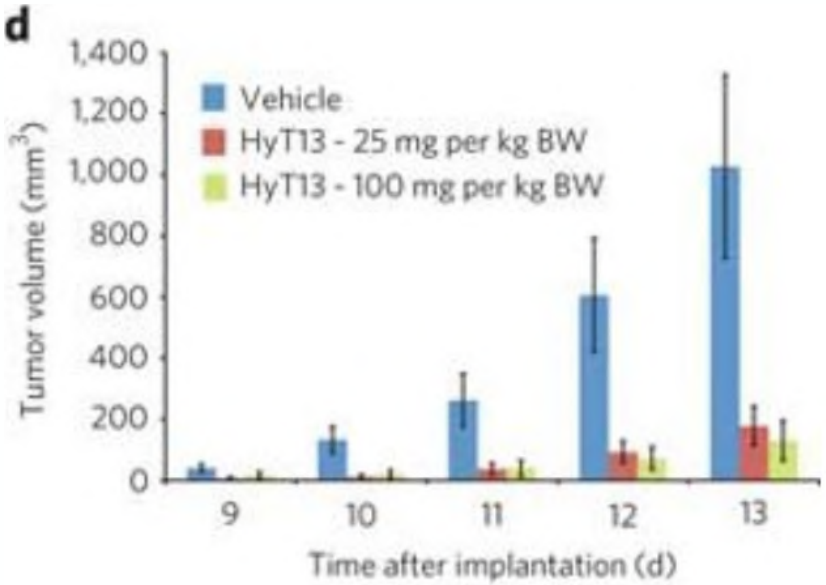
Neklesa, T.K. *et al. Nat. Chem. Biol.* **2011**, *7*, 538–543.

Hydrophobic Tagging (HyT)



Mouse xenographed with
NIH-3T3 cells expressing
HA–HaloTag-Hras1

Injected HyT13



HyT suppresses HaloTag-HRas1 driven tumor by degradation

Successful demontration of hydrophobic tagging leads to protein degradation

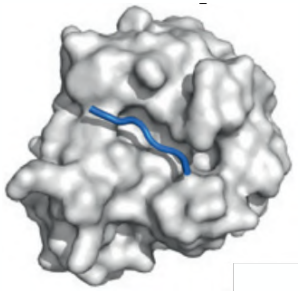
Covalent tagging method requires high dose

Protein degradation with alternative mechanism would have higher therapeutic potential

Neklesa, T.K. *et al. Nat. Chem. Biol.* **2011**, *7*, 538–543.

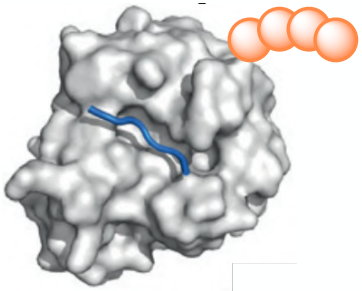
Proteolysis Targeting Chimera (PROTAC)

Ubiquitin–proteasome system

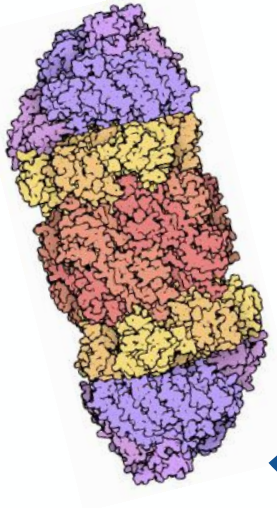


Target protein

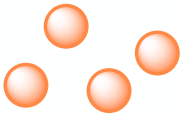
Ubiquitination by
E1, E2, E3 ligases



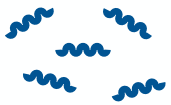
Ubiquitinated protein



Proteasome

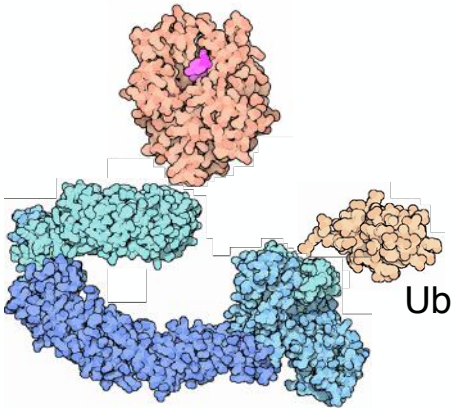


Ubiquitin



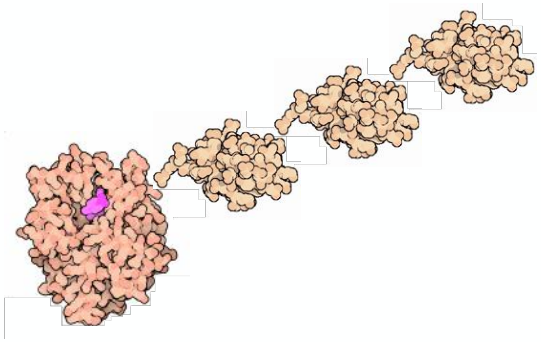
Small peptide

Target protein



Ub

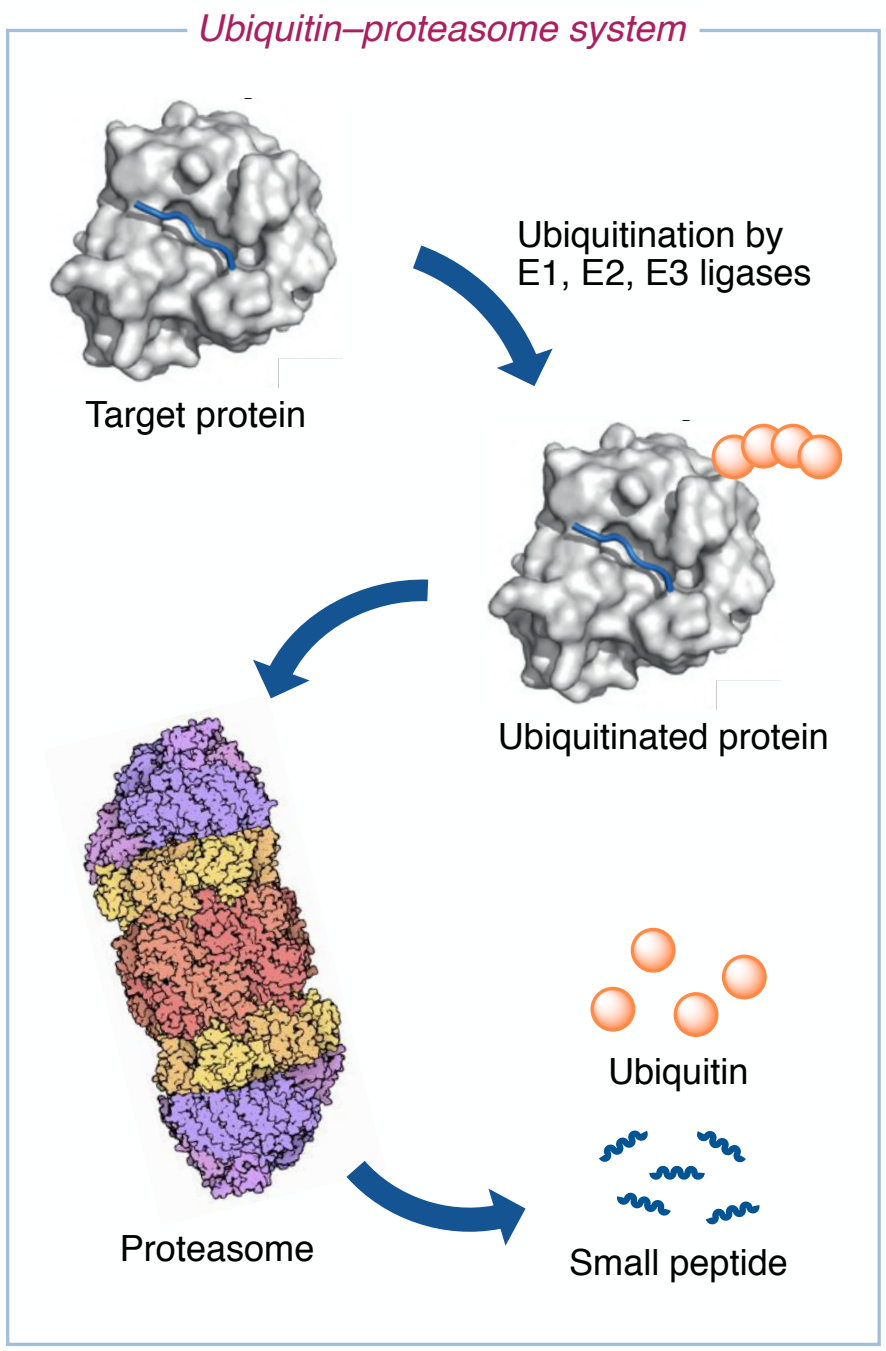
E3 ligase

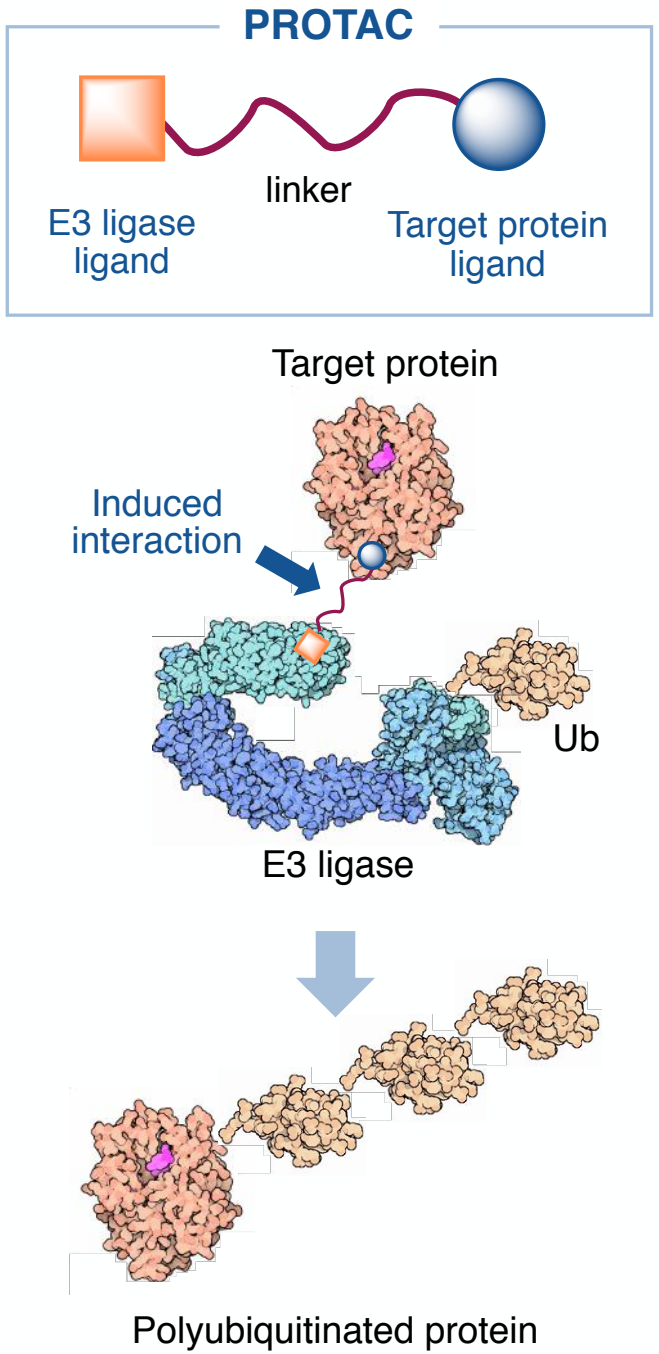


Polyubiquitinated protein

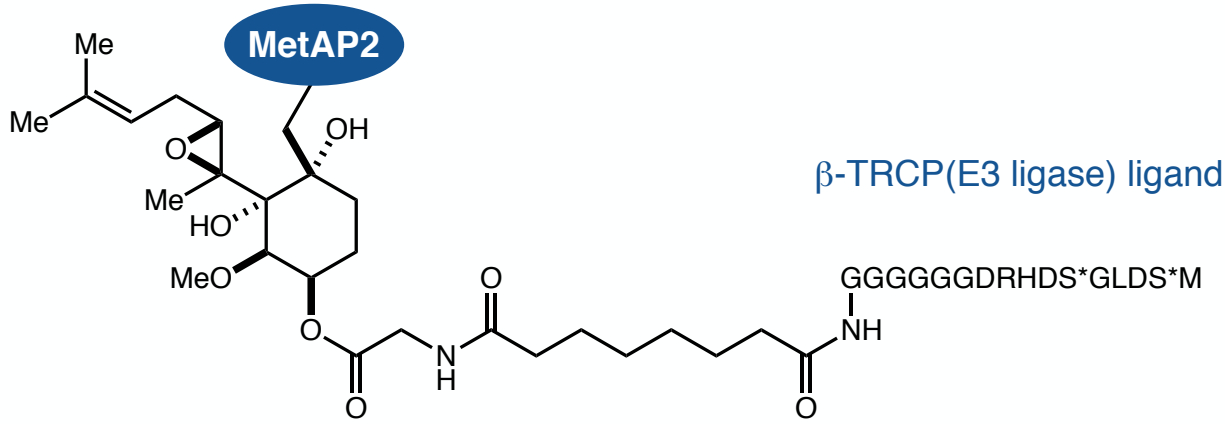
Can we induce artificial interaction
between E3 ligase and POI?

Proteolysis Targeting Chimera (PROTAC)



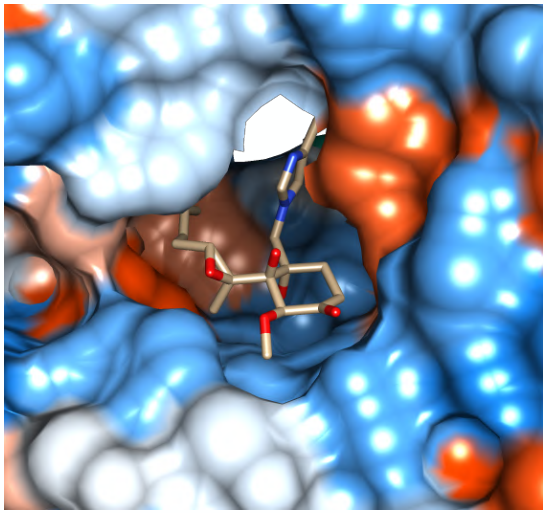


Proof of Concept



β-TRCP(E3 ligase) ligand

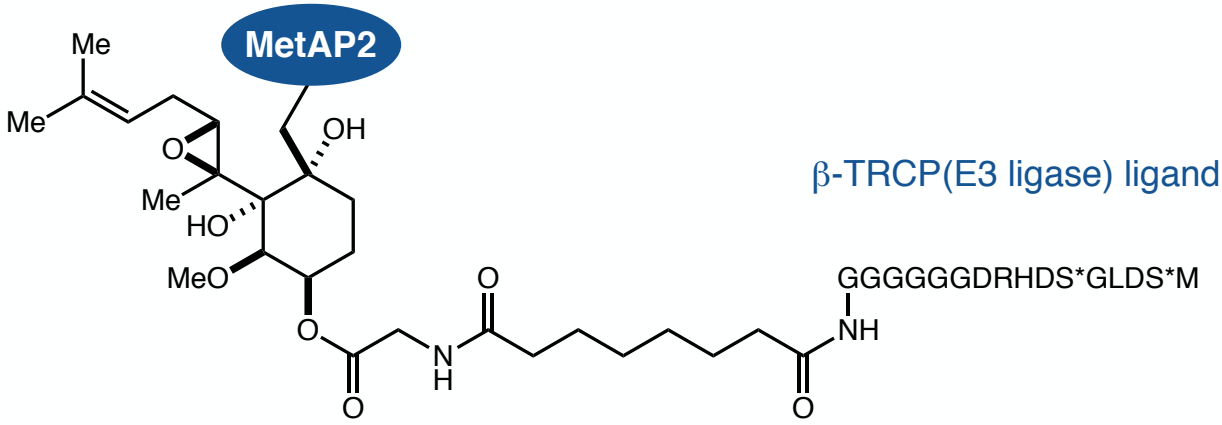
Ovalicin
MetAP2 covalent inhibitor



Covalently bound ovalicin
to MetAP2 (PDB:1B59)

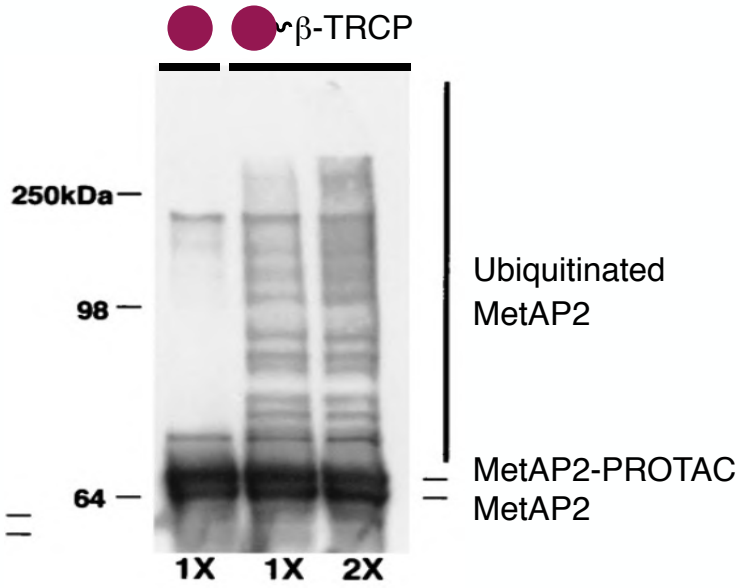
Sakamoto, K.M.; Kim, K.B.; Kumagai, A.; Mercurio, F.; Crews, C.M.; Deshaies, R.J. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, 98, 8554–8559.

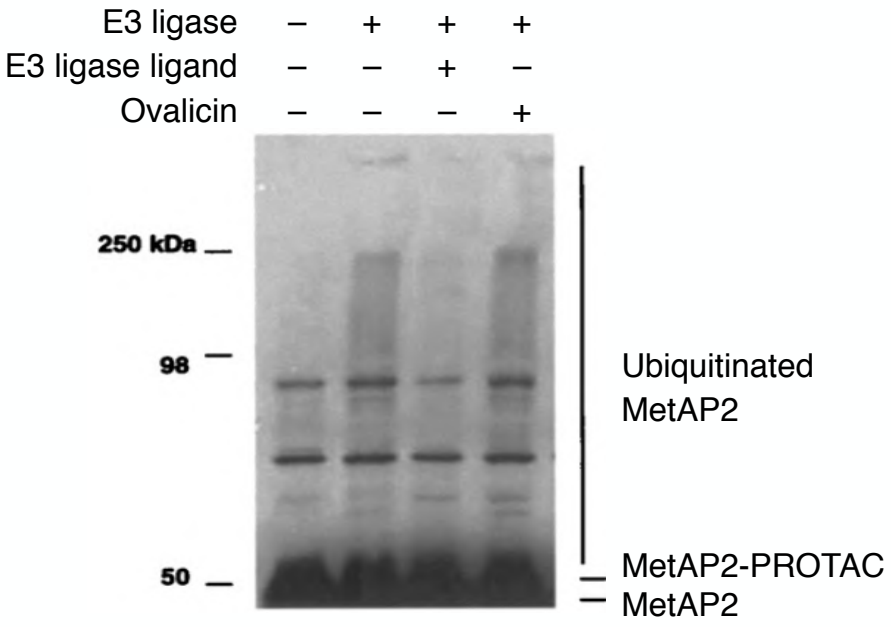
Proof of Concept



β-TRCP(E3 ligase) ligand

ATP, ubiquitin
E1, E2 enzymes





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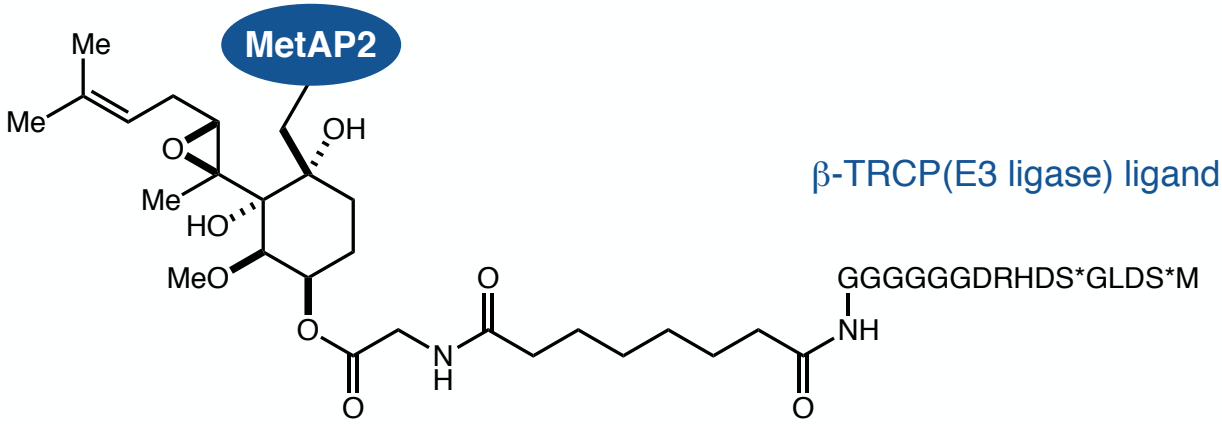
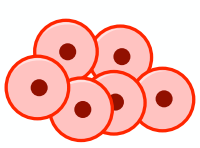
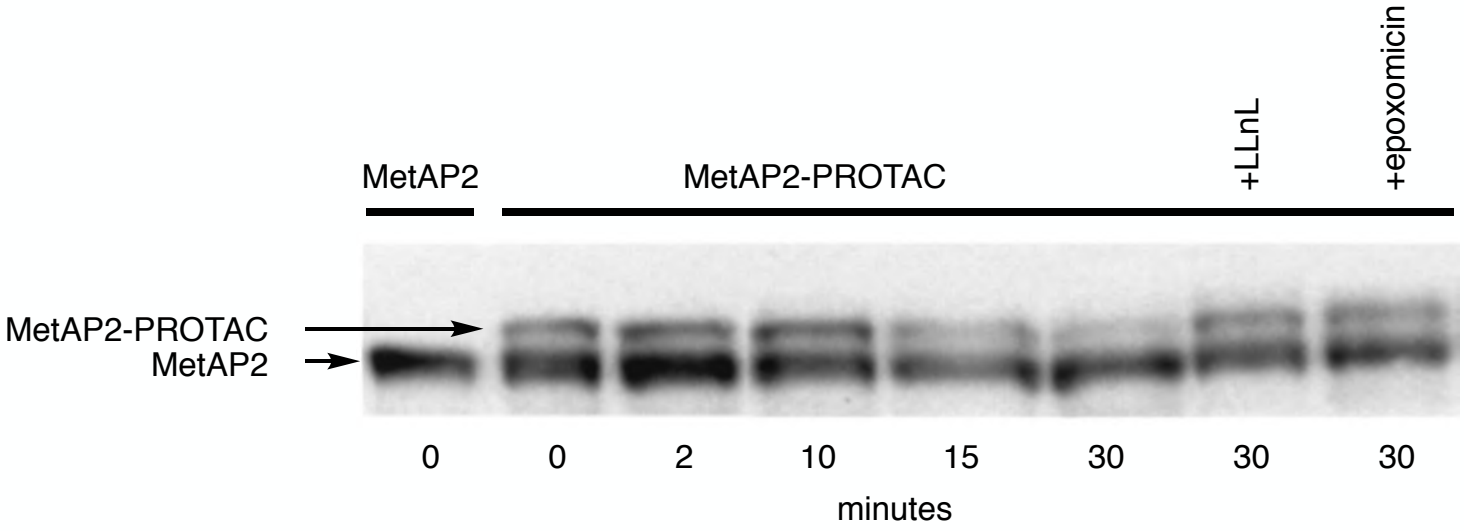




Frog egg extract



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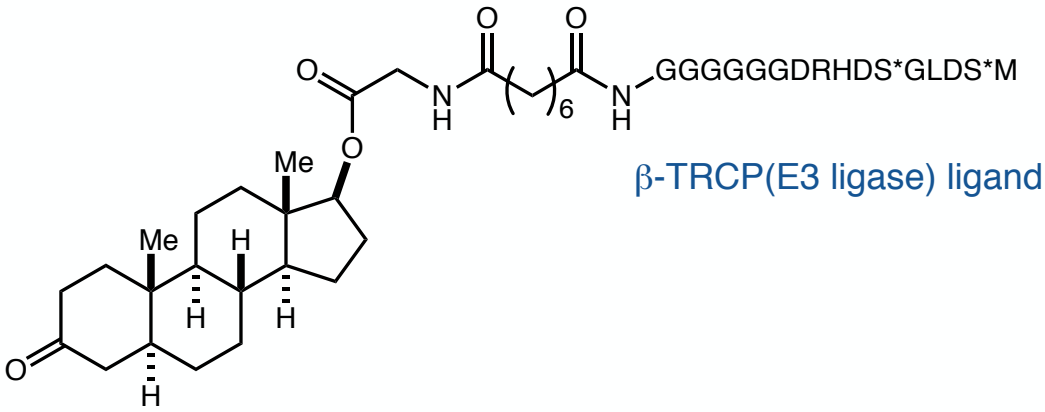
- Would it target therapeutically relevent targets?
  - Would it operate with noncovalent ligands?
    - Would it operate inside cells?

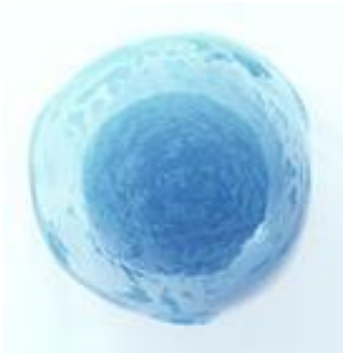
Intracellular Validation of PROTAC



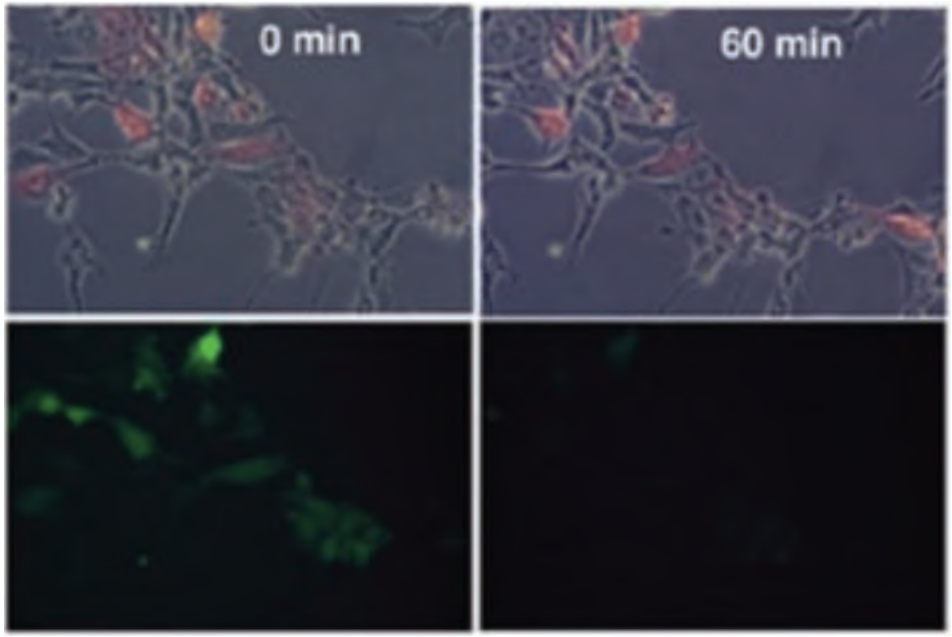
β-TRCP(E3 ligase) ligand

Dihydrotestosterone
Androgen receptor (AR) ligand

Microinjection



Cells expressing GFP–AR



>70% cells showed partial/complete loss of fluorescence

Sakamoto, K.M.; Kim, K.B.; Verma, R.; Ransick, A.; Stein, B.; Crews, C.M.; Deshaies, R.J. *Mol. Cell Proteomics* **2003**, *2*, 1350–1358.

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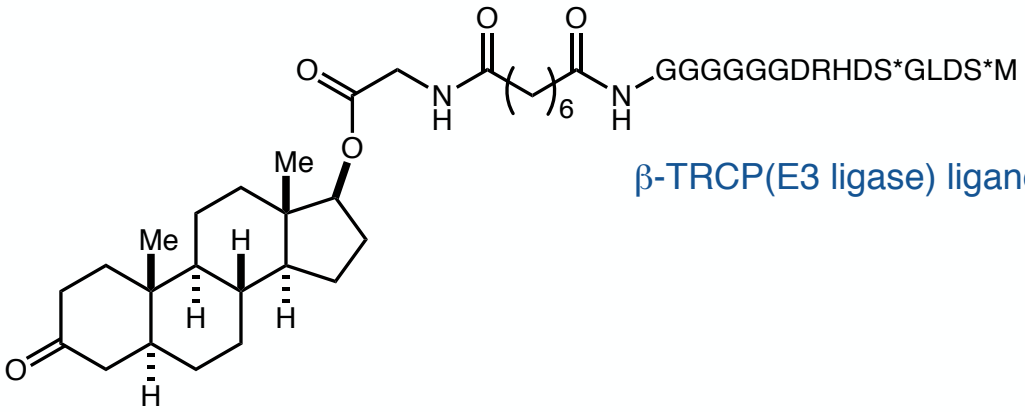

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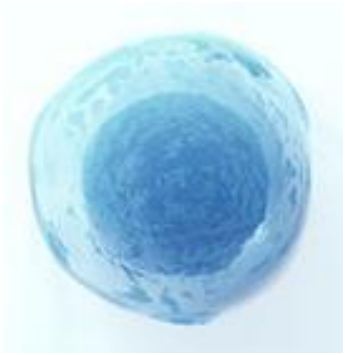
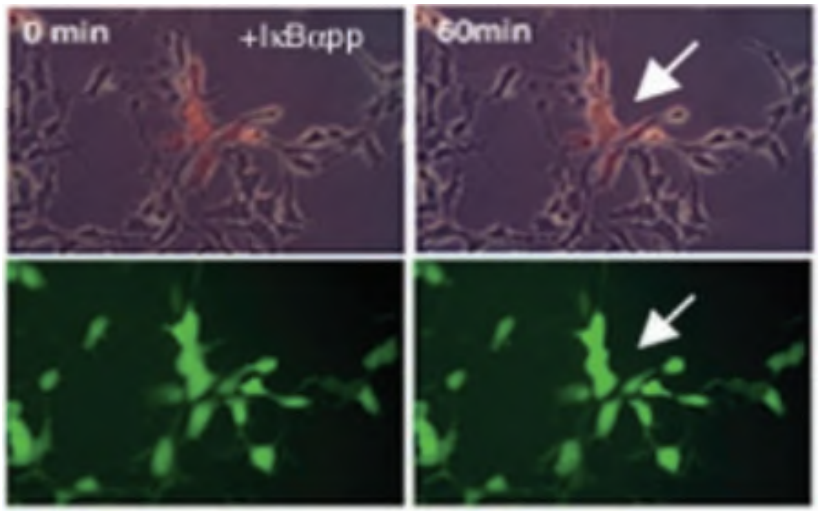
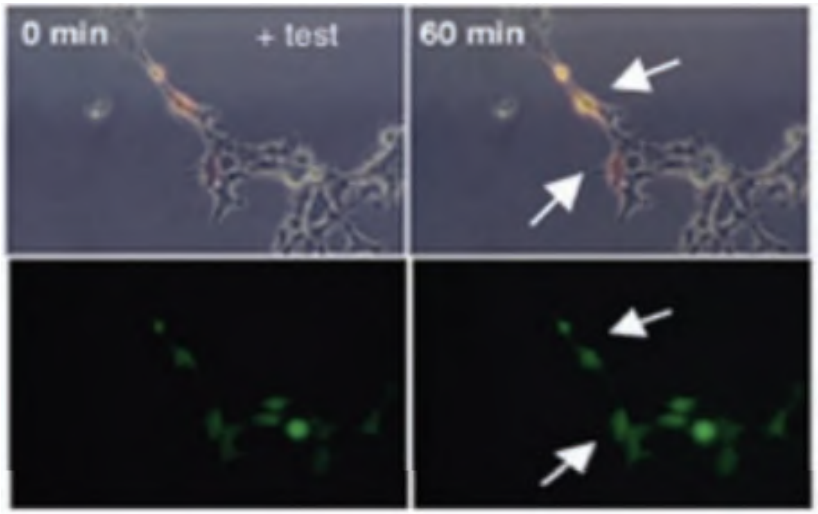


Cells expressing GFP–AR

PROTAC + E3 ligase ligand



PROTAC + testosterone



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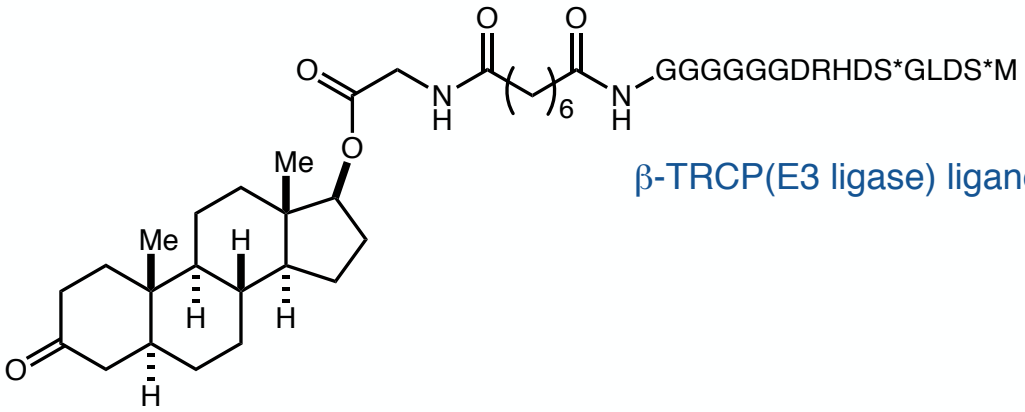
Intracellular Validation of PROTAC



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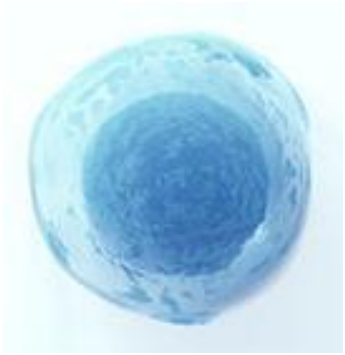
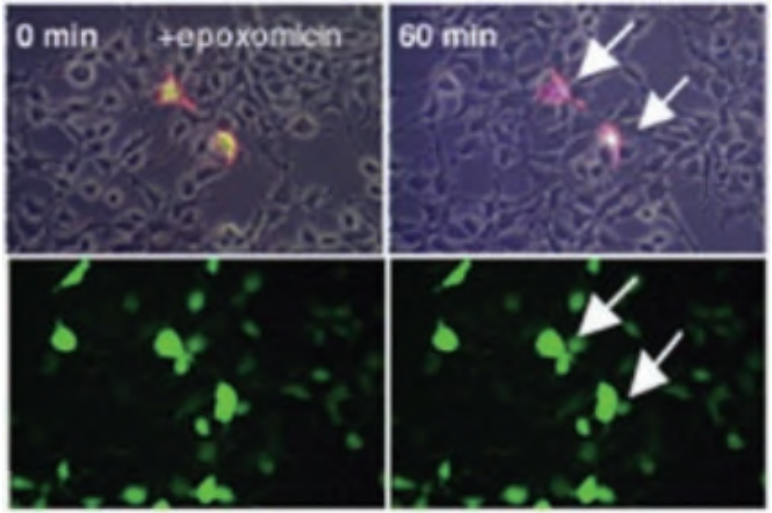


Cells expressing GFP–AR

PROTAC + proteasome inhibitor



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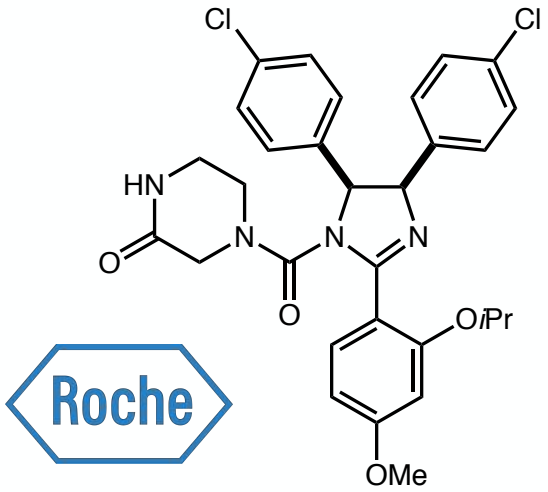
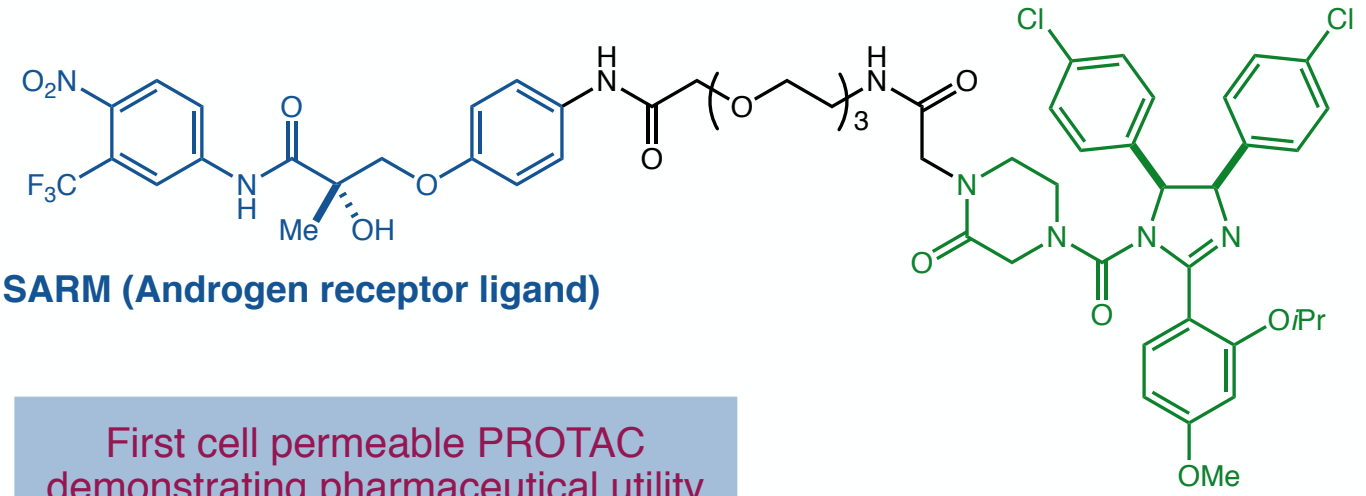
Small Molecule PROTAC

Peptide PROTAC

- Poor pharmacokinetics
- Poor cell permeability
- Poor intracellular stability
- Not amenable for high-throughput screening



Nutlin-3 (MDM2 inhibitor)
Science **2004**, 844.



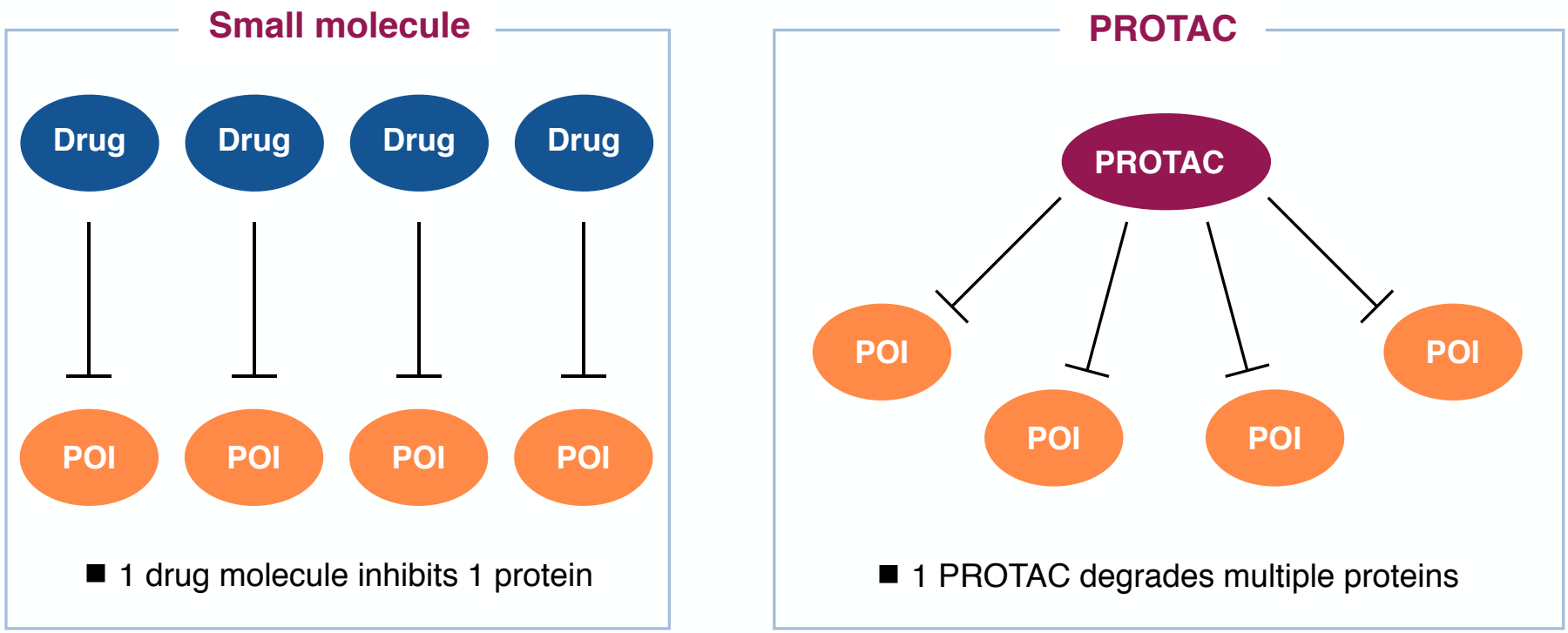
SARM (Androgen receptor ligand)

Nutlin-3 (MDM2 ligand)

First cell permeable PROTAC demonstrating pharmaceutical utility

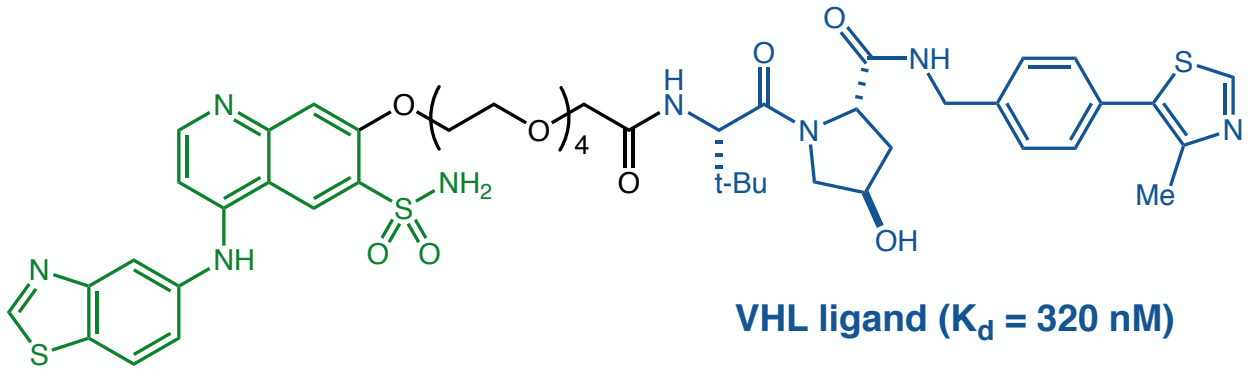
Schneekloth, A.R.; Pucheault, M.; Tae, H.S.; Crews, C.M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5904–5908.

Small Molecule Drugs vs. PROTAC



Catalytic mode of action can provide high potency and selectivity

Small Molecule Drugs vs. PROTAC

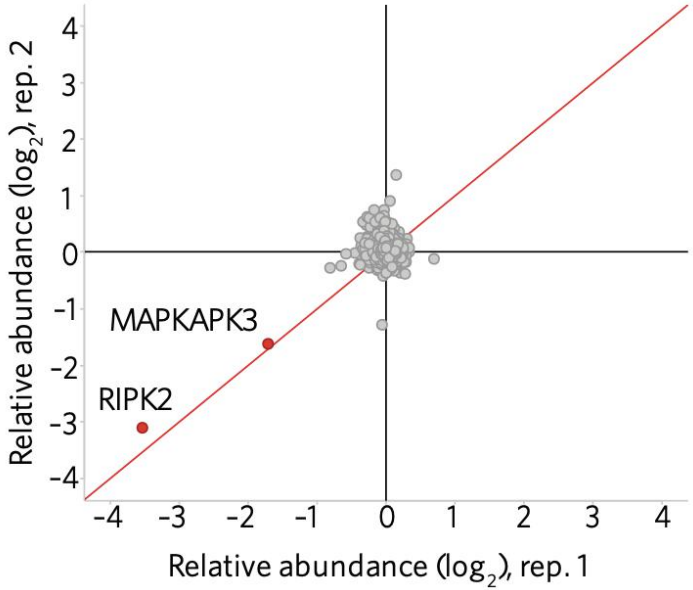


RIPK2 ligand (IC_{50} = 660 nM)

VHL ligand (K_d = 320 nM)

DC_{50} = 1.4 nM

DC_{max} = 10 nM

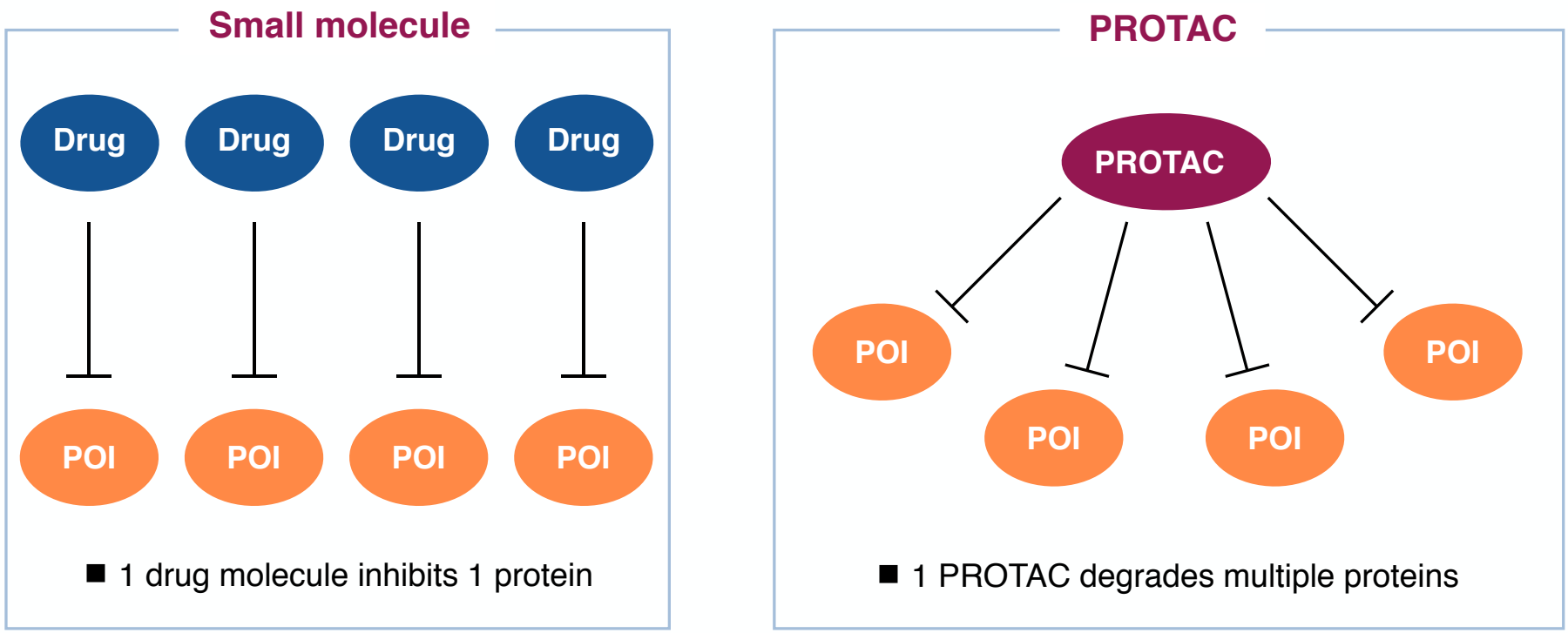


Only RIPK2 and MAPKAPK3

were degraded among 7640 proteins

Bondeson, D.P.; Mares, A. *et al. Nat. Chem. Biol.* **2015**, *11*, 611–617.

Small Molecule Drugs vs. PROTAC

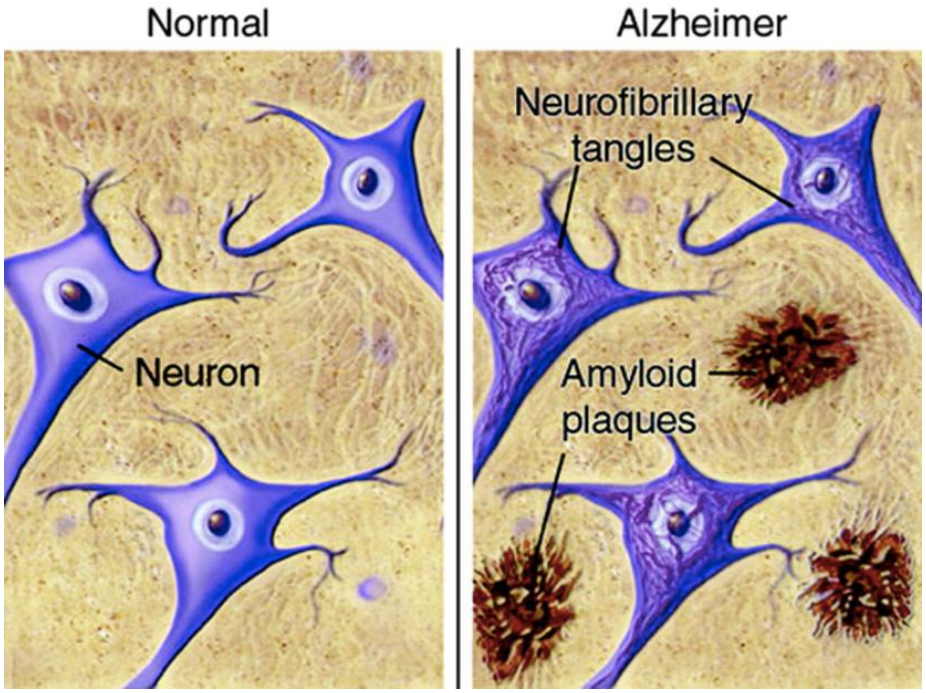


Catalytic mode of action can provide high potency and selectivity

Only affinity probes are required – no need to be inhibitors

Removal of a protein instead of inhibition can provide additional therapeutic effect

Potential Application of PROTAC in Neurodegenerative Diseases

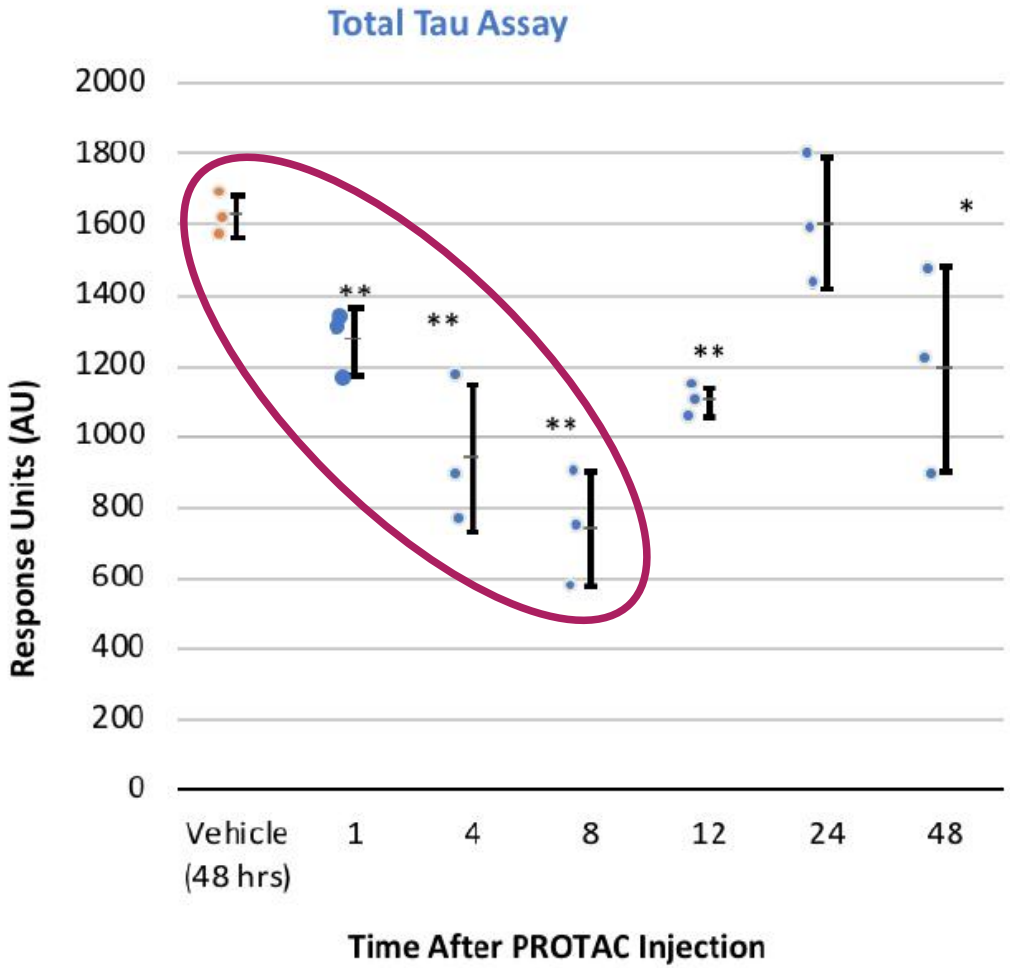


Accumulation of misfolded proteins (tau, β-amyloid) is suspected to cause neurodegenerative diseases

Could misfolded proteins be removed via PROTAC?

Harding, R.H.; Tong, Y. *Acta Pharmacol. Sin.* **2018**, *39*, 754–769.

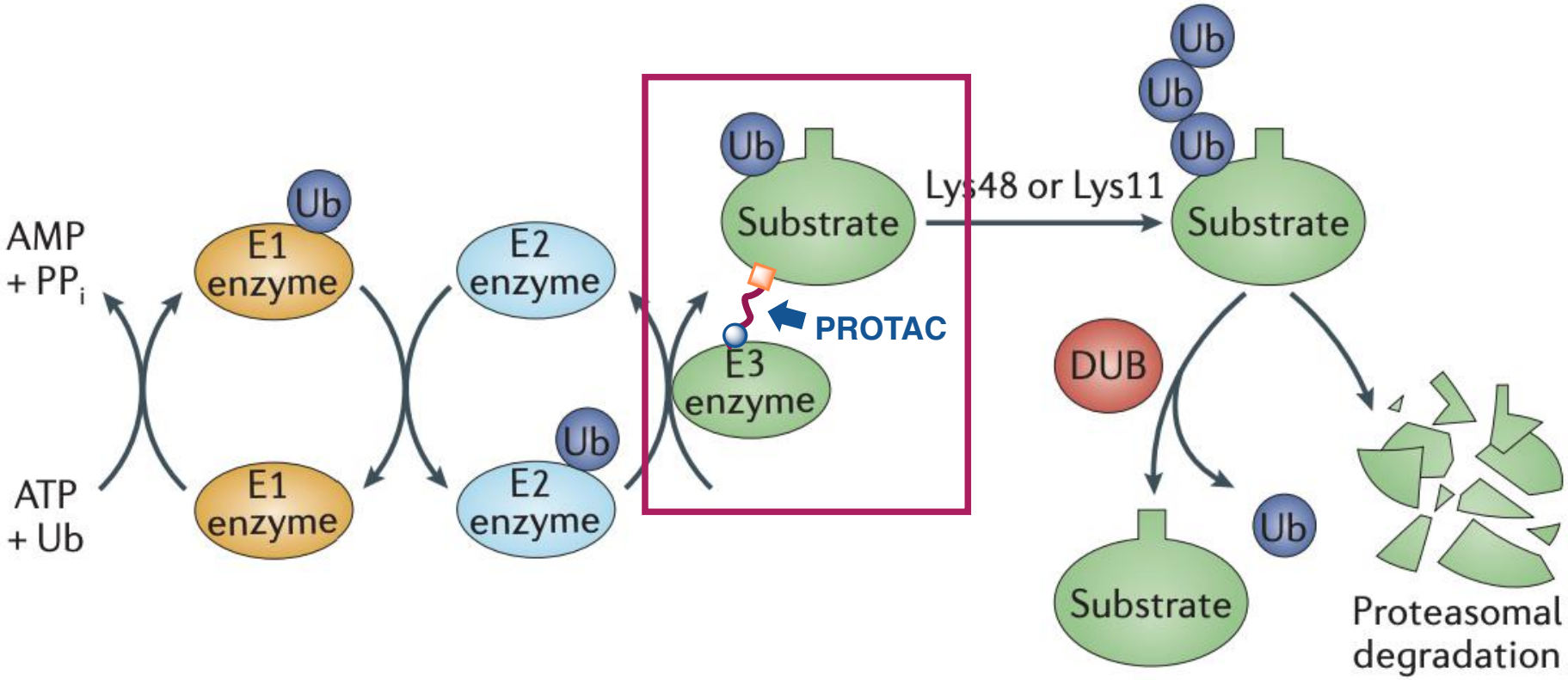
Potential Application of PROTAC in Neurodegenerative Diseases



Direct injection of tau-directed PROTAC reduced tau levels by 50%

http://arvinas.com/therapeutic-programs/next-generation-targets/

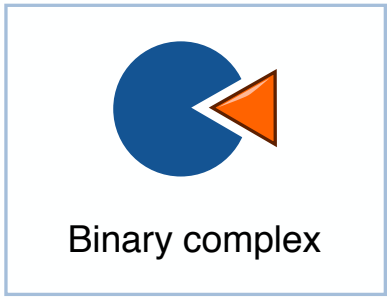
What Problems Are Left for PROTACs?



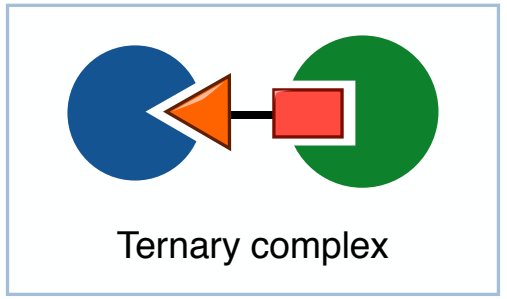
Efficient ternary structure formation and ubiquitin transfer

Skaar, J.R.; Pagan, J.K.; Pagano, M. *Nat. Rev. Drug Disc.* **2014**, *13*, 889–903.

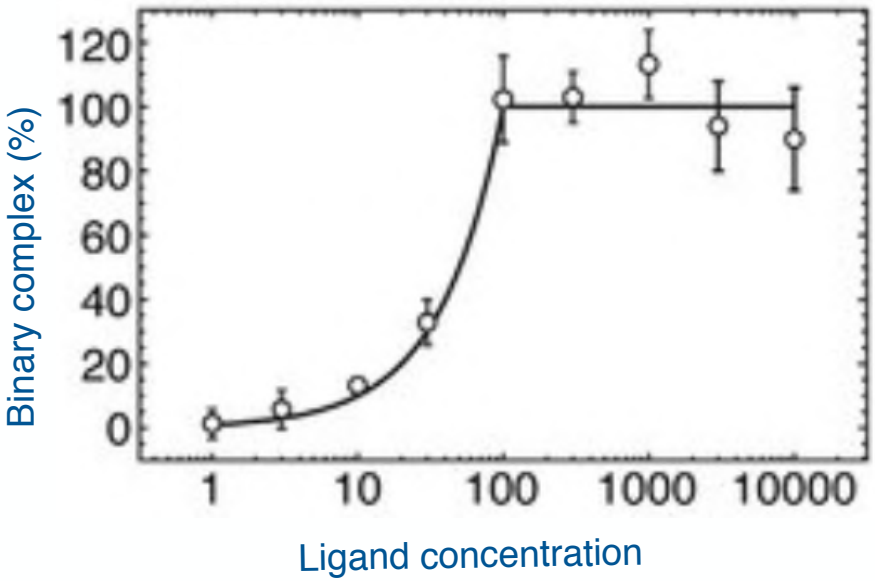
What Problems Are Left for PROTACs?

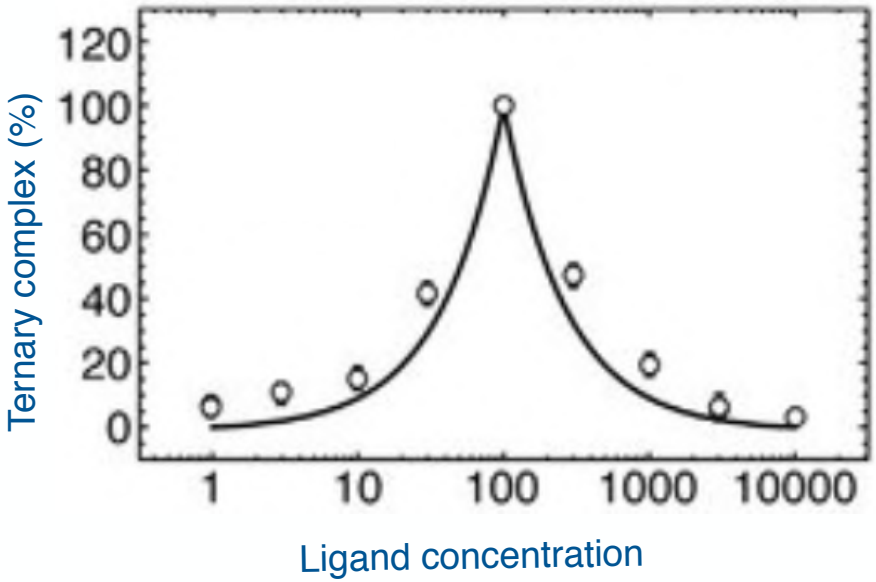


Binary complex



Ternary complex

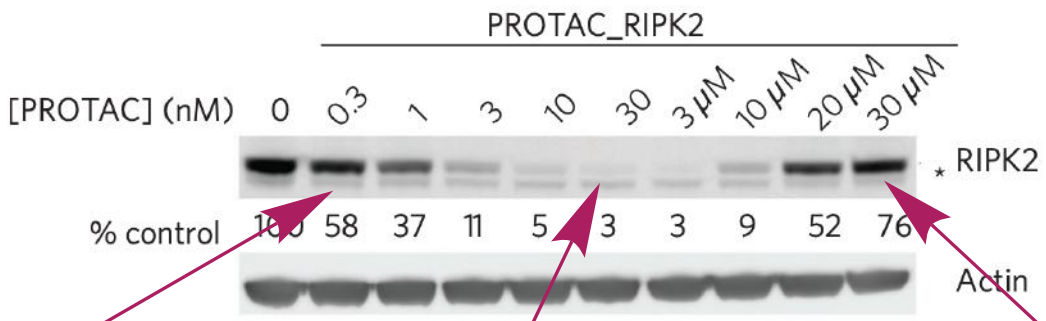


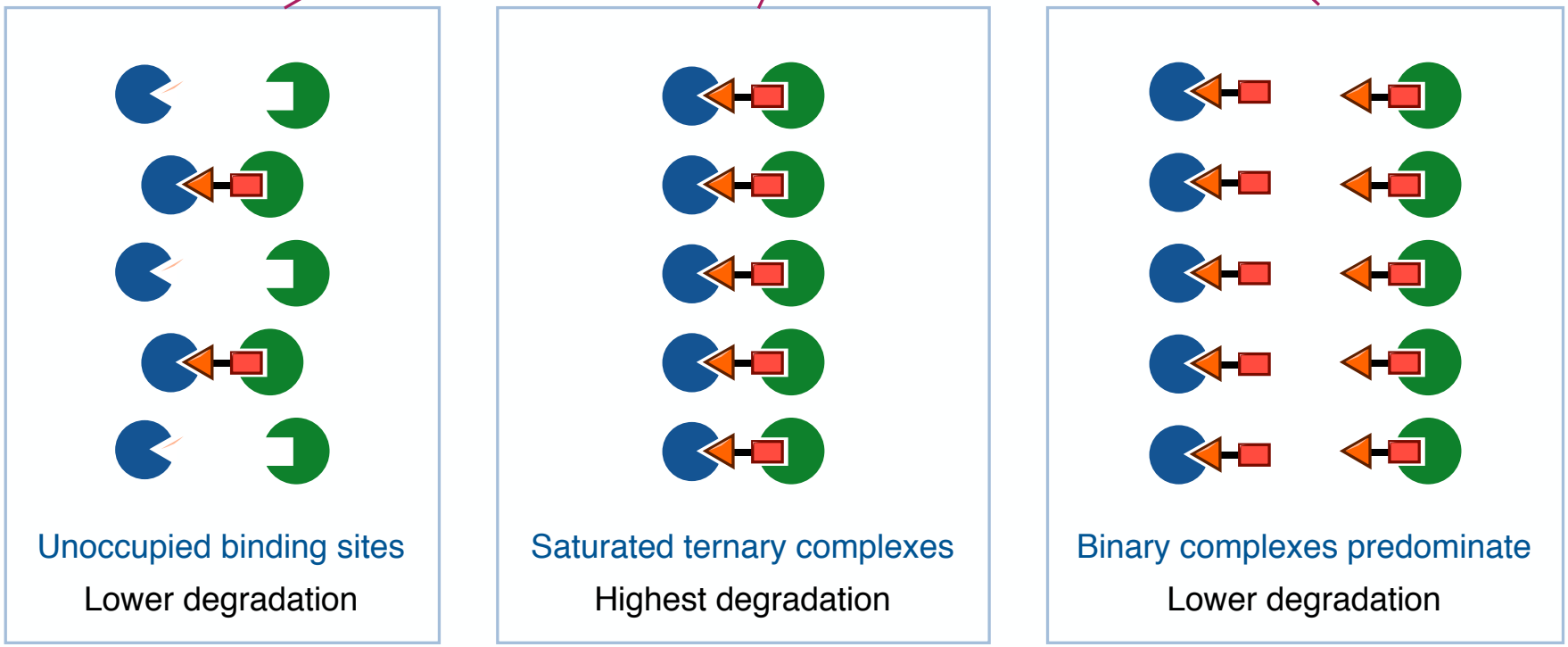


Hook effect – increasing the concentration of bisligand can decrease ternary complex concentration

Douglass, E.F.; Miller, C.J.; Sparer, G.; Shapiro, H.; Spiegel, D.A. *J. Am. Chem. Soc.* **2013**, *135*, 6092–6099.

What Problems Are Left for PROTACs?





Unoccupied binding sites
Lower degradation

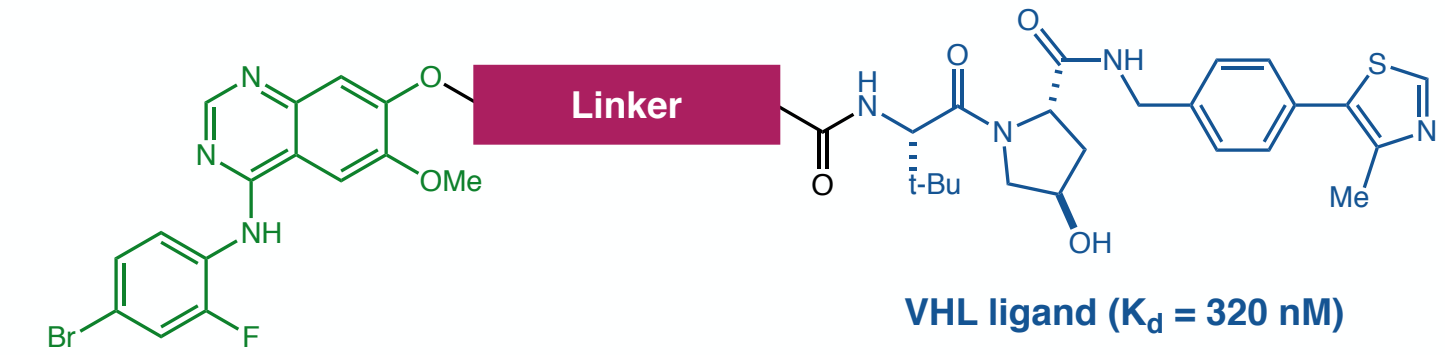
Saturated ternary complexes
Highest degradation

Binary complexes predominate
Lower degradation

Matching the right E3 ligase/ligand – target protein/ligand combination is crucial for efficient degradation

Bondeson, D.P.; Mares, A. *et al. Nat. Chem. Biol.* **2015**, *11*, 611–617.

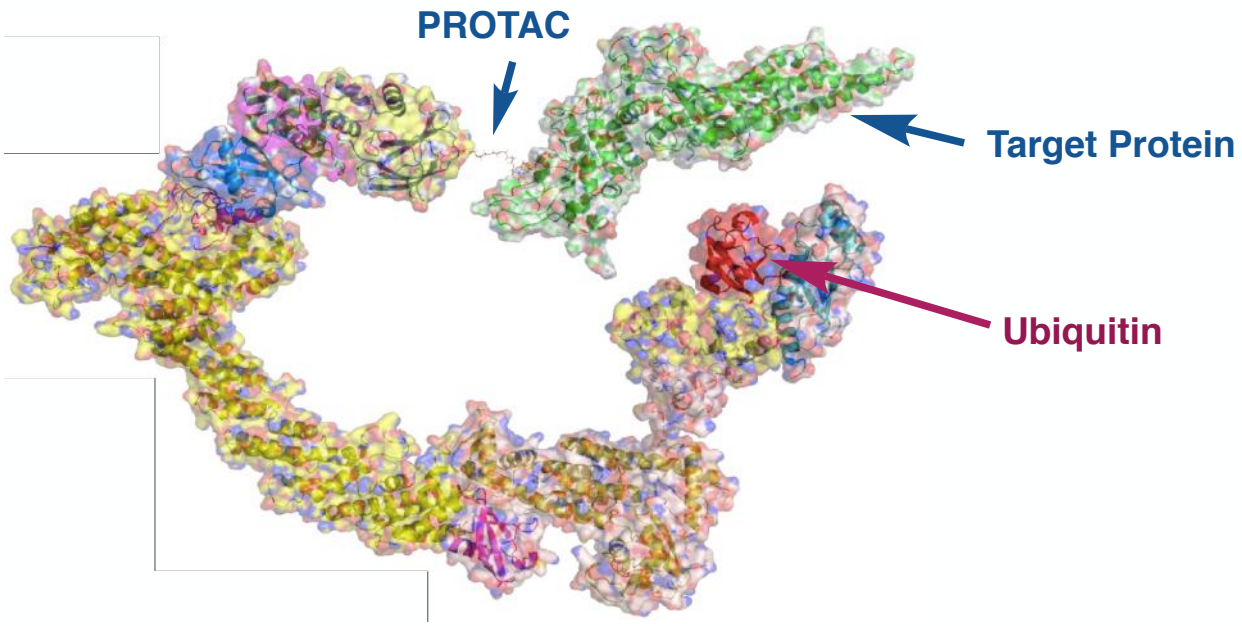
What Problems Are Left for PROTACs?



VEGFR2 inhibitor (IC_{50} = 40 nM)

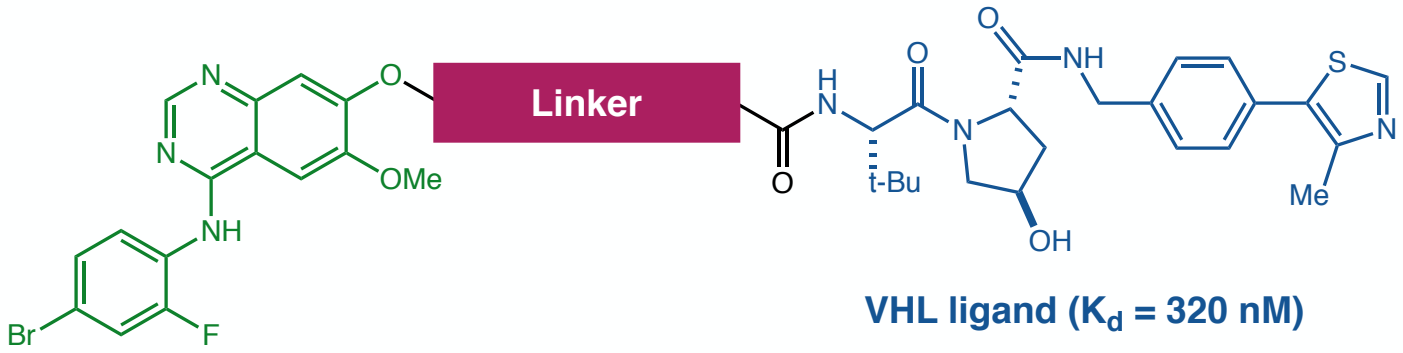
VHL ligand (K_d = 320 nM)

What linker structure facilitate ternary complex formation and ubiquitination?



Bondeson, D.P.; Mares, A. *et al. Nat. Chem. Biol.* **2015**, *11*, 611–617.

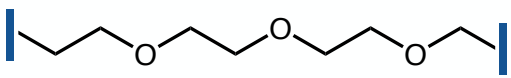
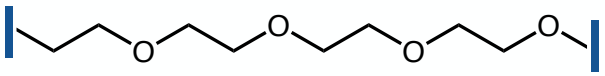
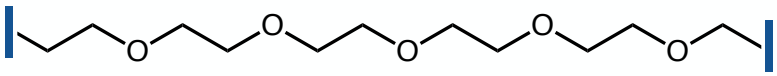
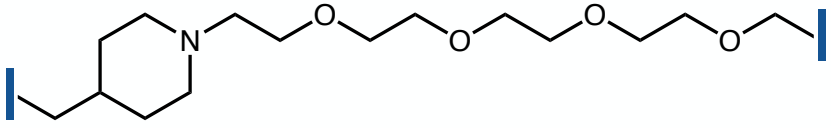
What Problems Are Left for PROTACs?



VEGFR2 inhibitor (IC_{50} = 40 nM)

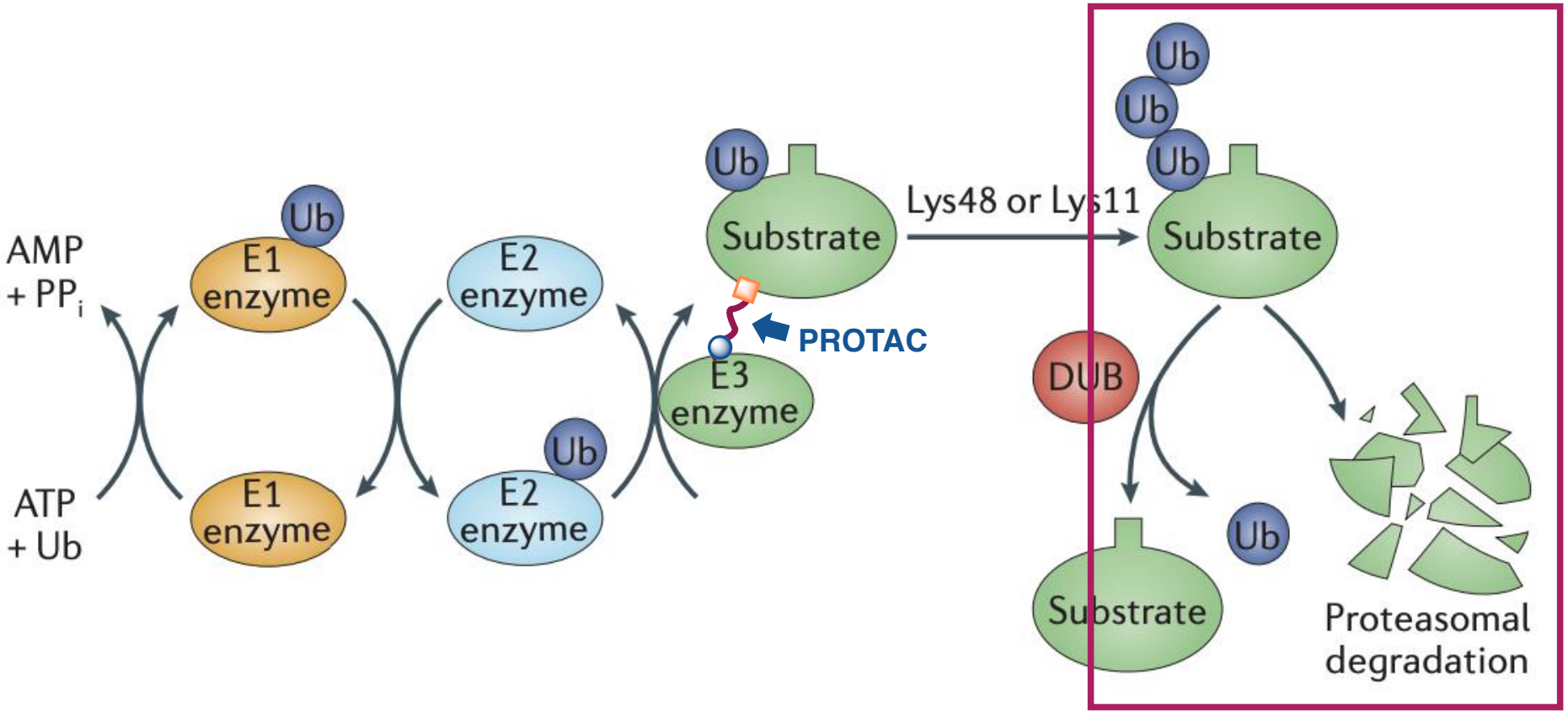
VHL ligand (K_d = 320 nM)

What linker structure facilitate ternary complex formation and ubiquitination?

Linker	# of atoms	DC_{50}
	10	2 μM
	12	0.8 μM
	16	No effect upto 3 μM
	18	Weak effect at 10 μM

Bondeson, D.P.; Mares, A. *et al. Nat. Chem. Biol.* **2015**, *11*, 611–617.

What Problems Are Left for PROTACs?



Efficient ternary structure formation and ubiquitin transfer

Rate of degradation should be faster than rate of synthesis

Skaar, J.R.; Pagan, J.K.; Pagano, M. *Nat. Rev. Drug Disc.* **2014**, *13*, 889–903.

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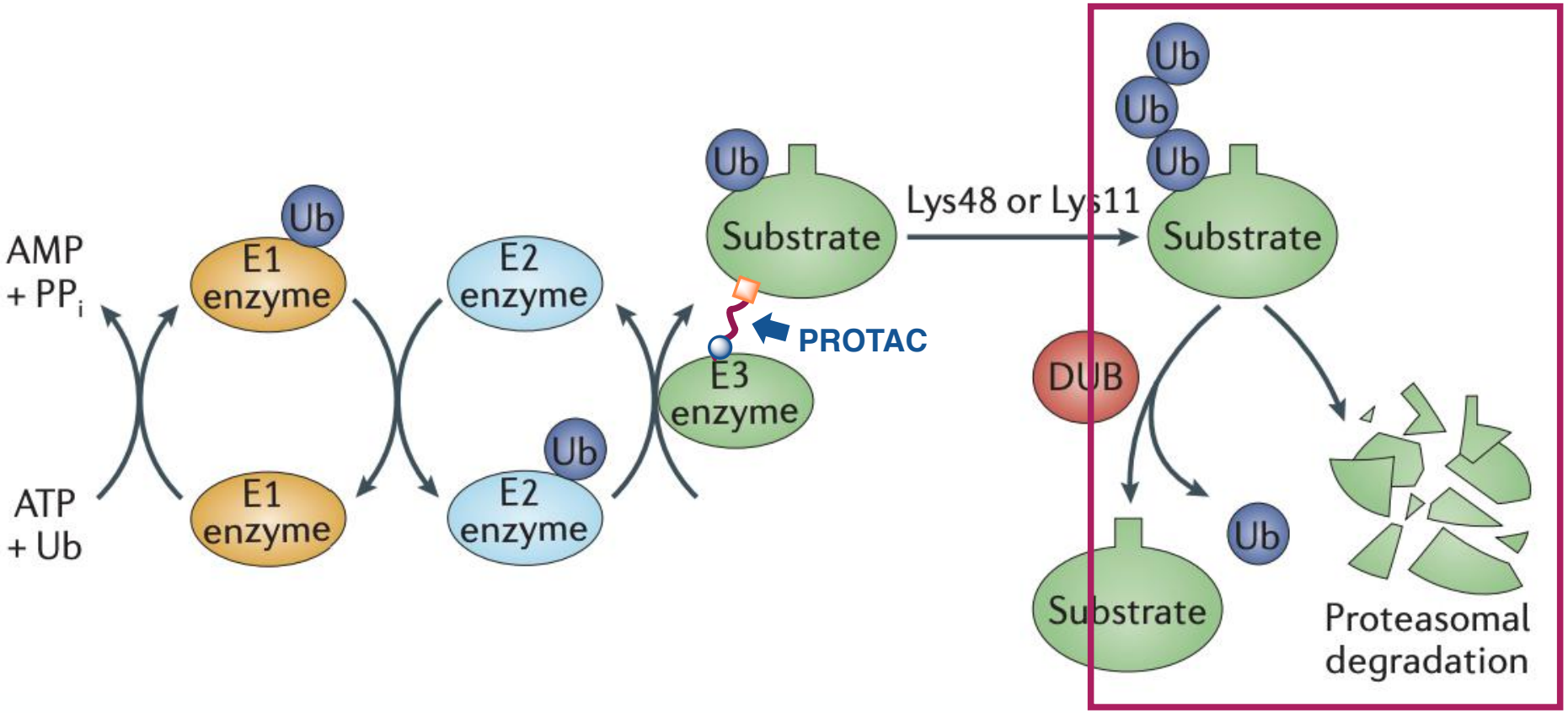
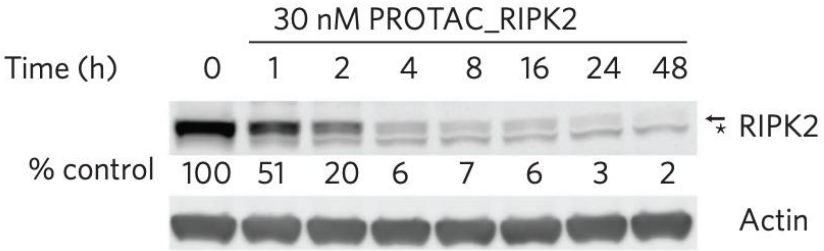
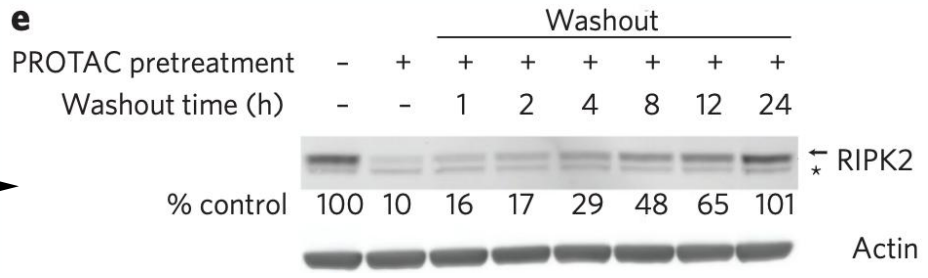




Washout



Skaar, J.R.; Pagan, J.K.; Pagano, M. *Nat. Rev. Drug Disc.* **2014**, *13*, 889–903.

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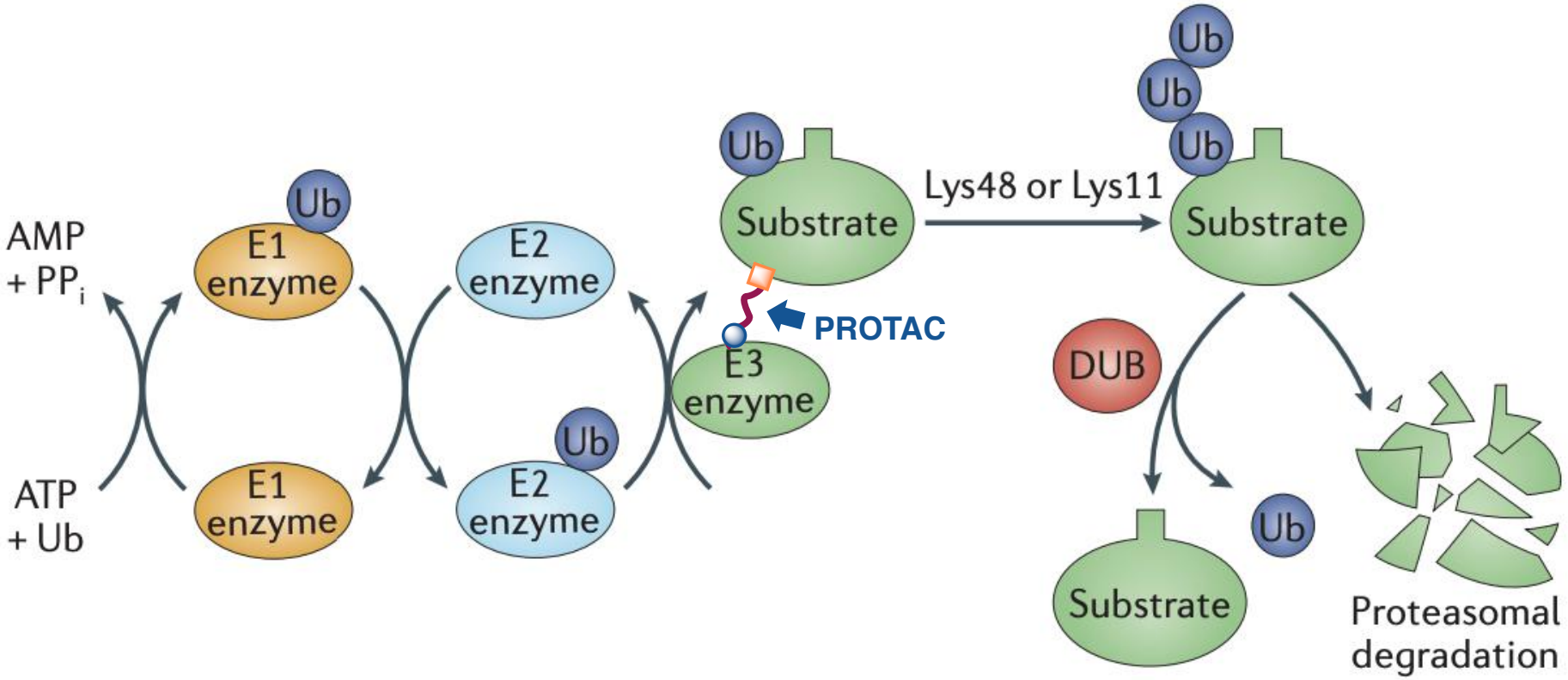

Efficient ternary structure formation and ubiquitin transfer

Rate of degradation should be faster than rate of synthesis

A series of processes must be orchestrated for efficient protein degradation

Skaar, J.R.; Pagan, J.K.; Pagano, M. *Nat. Rev. Drug Disc.* **2014**, *13*, 889–903.