Conditional control of protein degradation with small molecule

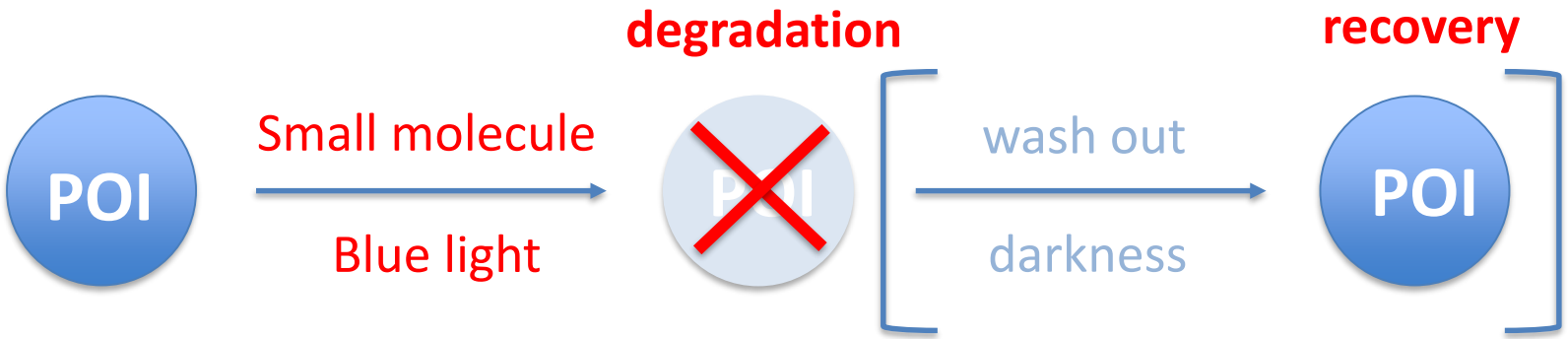
2017.10.21

M2 Yamaji Kyohei

Contents

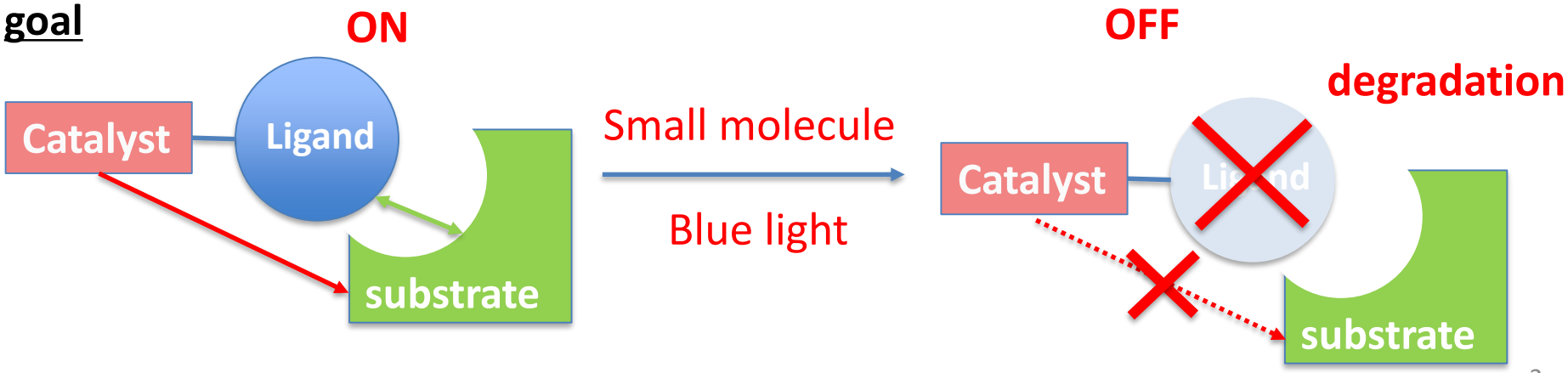
Overview of the technology and future application

Chemical tools to degrade and recover the protein of interest artificially



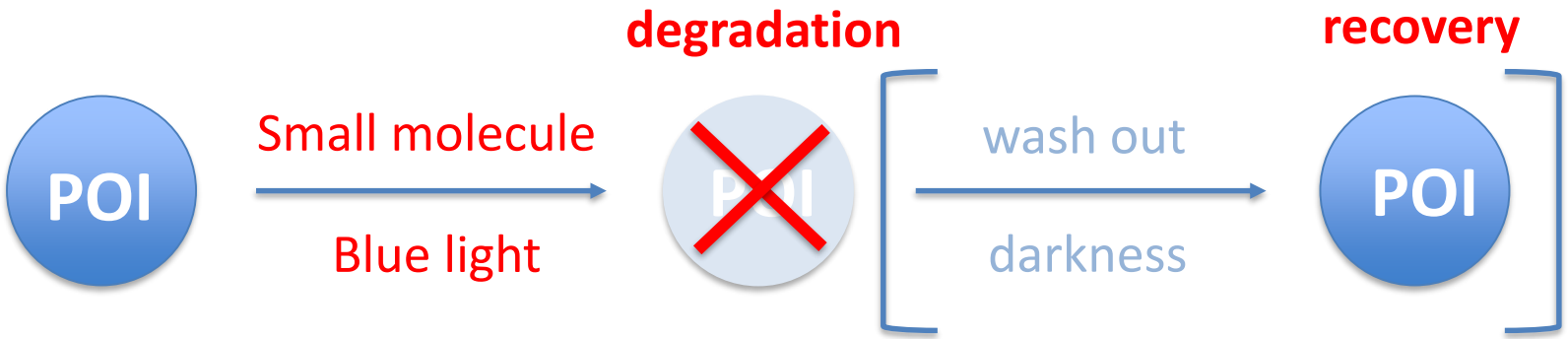
How can this technology be applied to Catalysis medicine?

On/Off Switching System of the Catalyst with artificial stimuli



Two strengths of the technology

Chemical tools to degrade and recover the protein of interest artificially



Why was this technology invented?

✔**Powerful tool for biological research**

✔**new concept of potent drug**

Tools to control protein degradation conditionally

No Genetic manipulation

1st generation PROTACs

Dr. Crews

2001 Protac-1

peptide

2004 Protac-4

2nd generation PROTACs

2008 MDM2based Small-molecule

2011 cIAP-1 based

VHL based

2015 CRBN based

HyT system

2011 Adamantane

2012 $(Boc)_3Arg$

Small-molecule

Genetic manipulation

Dr. Wandless

DD system based

2006 DD and Shld-1

Small-molecule

2011 LID system

Small-molecule

2013 B-LID system

Blue light

AID system

2009 AID system

Small-molecule

Dr. Kanemaki

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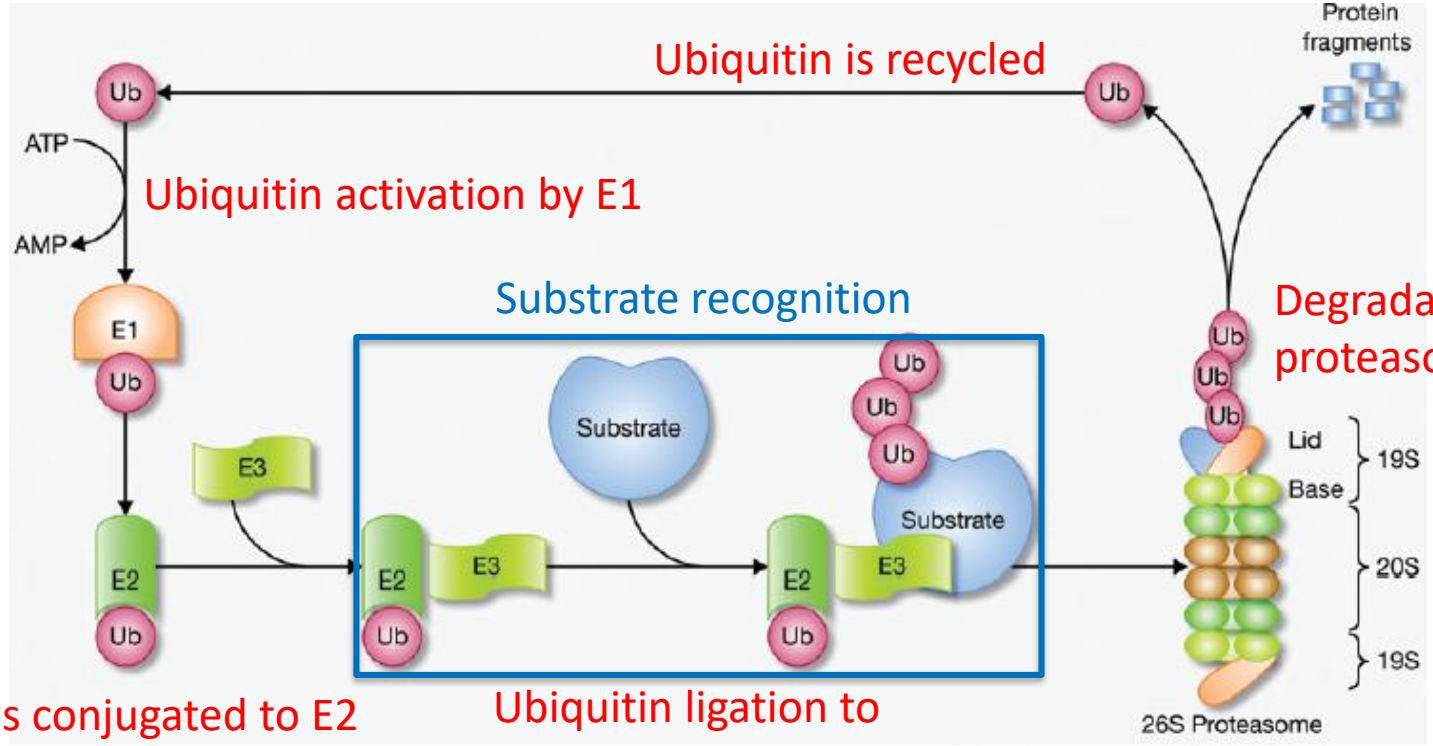
Small-molecule

Dr. Kanemaki

Ubiquitin/Proteasome System (UPS)

Ubiquitin/Proteasome System

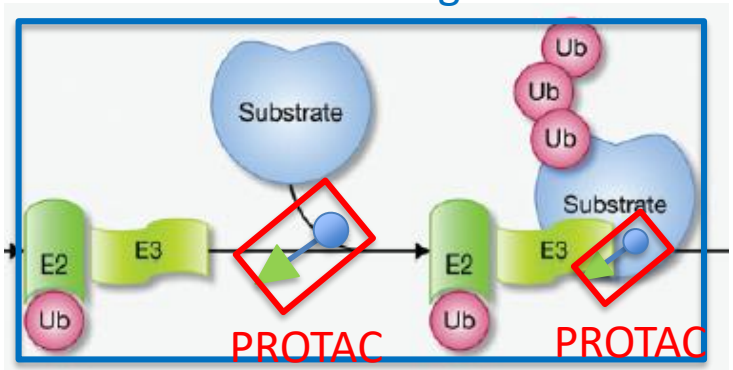


E1: ubiquitin-activating enzyme --- only 1 class in many organisms
E2: ubiquitin-conjugating enzyme--- several families
E3: ubiquitin ligase --- hundreds of kinds of ligases
→**substrate specificity**

Works of Craig Crews; PROTACs

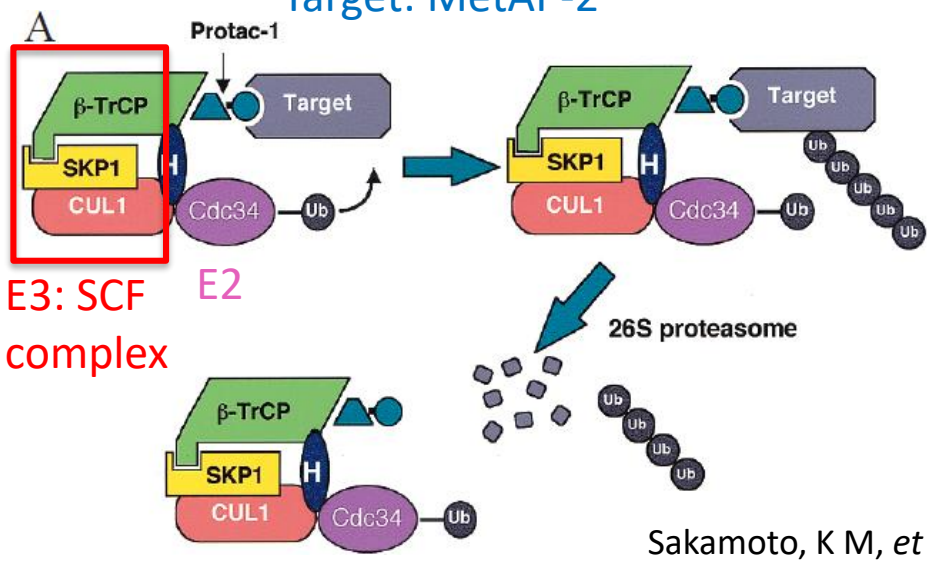
PROTACs bridges E3 ligase and substrate to promote ubiquitination

Substrate recognition



The design of first PROTAC

Target: MetAP-2

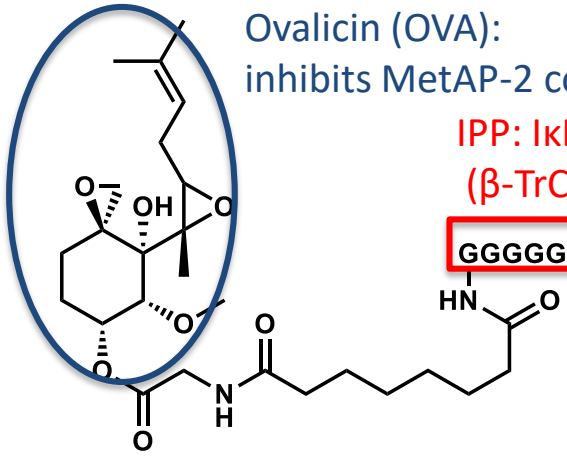


The structure of Protac-1

Ovalicin (OVA):
inhibits MetAP-2 covalently

IPP: IκBα phosphopeptide
(β-TrCP subtrate)

GGGGGGDRHD*p*SGLD*p*SM-COOH



Sakamoto, K M, *et al. Proc. Natl. Acad. Sci. U.S. A.* **2001**, *98*:8554

Short Summary of Peptidic PROTACs

No Genetic manipulation

Dr. Crews

1st generation PROTACs

2001 Protac-1

2004 Protac-4

peptide

✔proof-of-concept

✕High molecular weight

✕poor cell permeability

✕metabolic instability

small-molecule-induced protein degradation would be better

Genetic manipulation

Dr. Wandless

DD system based

2006 DD and Shld-1

Small-molecule

AID system

2009 AID system

Small-molecule

2011 LID system

Small-molecule

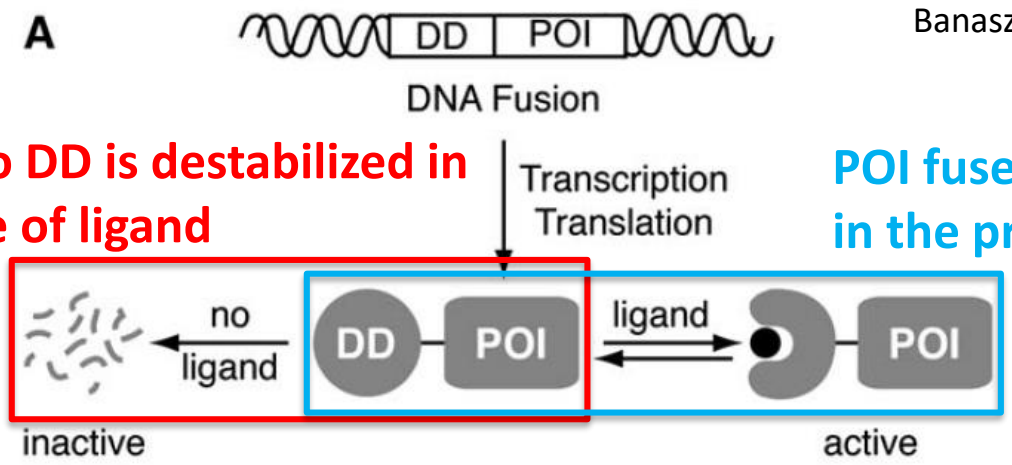
2013 B-LID system

Blue light

Dr. Kanemaki

artificial degron stabilized by small molecules

Concept of Small-molecule responsive destabilizing domains (DD)



Banaszynski, L. A., *et al. Cell,* **2006**, *126*:995

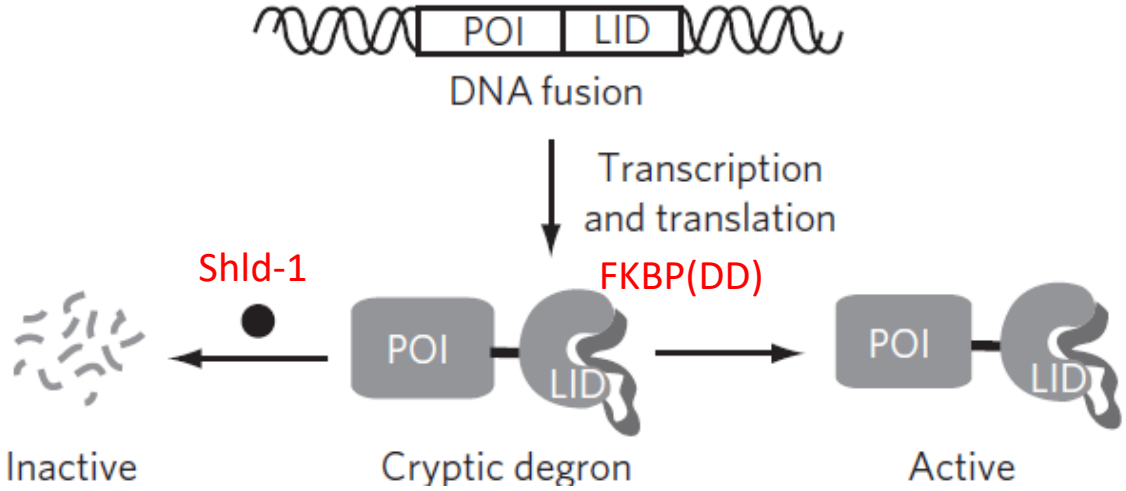
POI fused to DD is destabilized in the absence of ligand

POI fused to DD is stabilized in the presence of ligand

POI: protein of interest

DD: destabilizing domain

Concept of Ligand Inducible Degradation (LID) system



Short Summary of Dr. Wandless' work

No Genetic manipulation

✔high specificity
✔temporal and dose-dependent
✔recovery of protein
✔low toxicity

×genetic manipulation
×continued presence of Shld-1 (DD)

Small-molecule driven technology without genetic manipulation was pursued.

HyT system

2011 cIAP-1 based
VHL based
2011 Adamantane
2012 (Boc)3Arg
2015 CRBN based

Small-molecule

Genetic manipulation

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2nd generation PROTACs

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VHL based

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HyT system

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Small-molecule
2013 B-LID system
Blue light

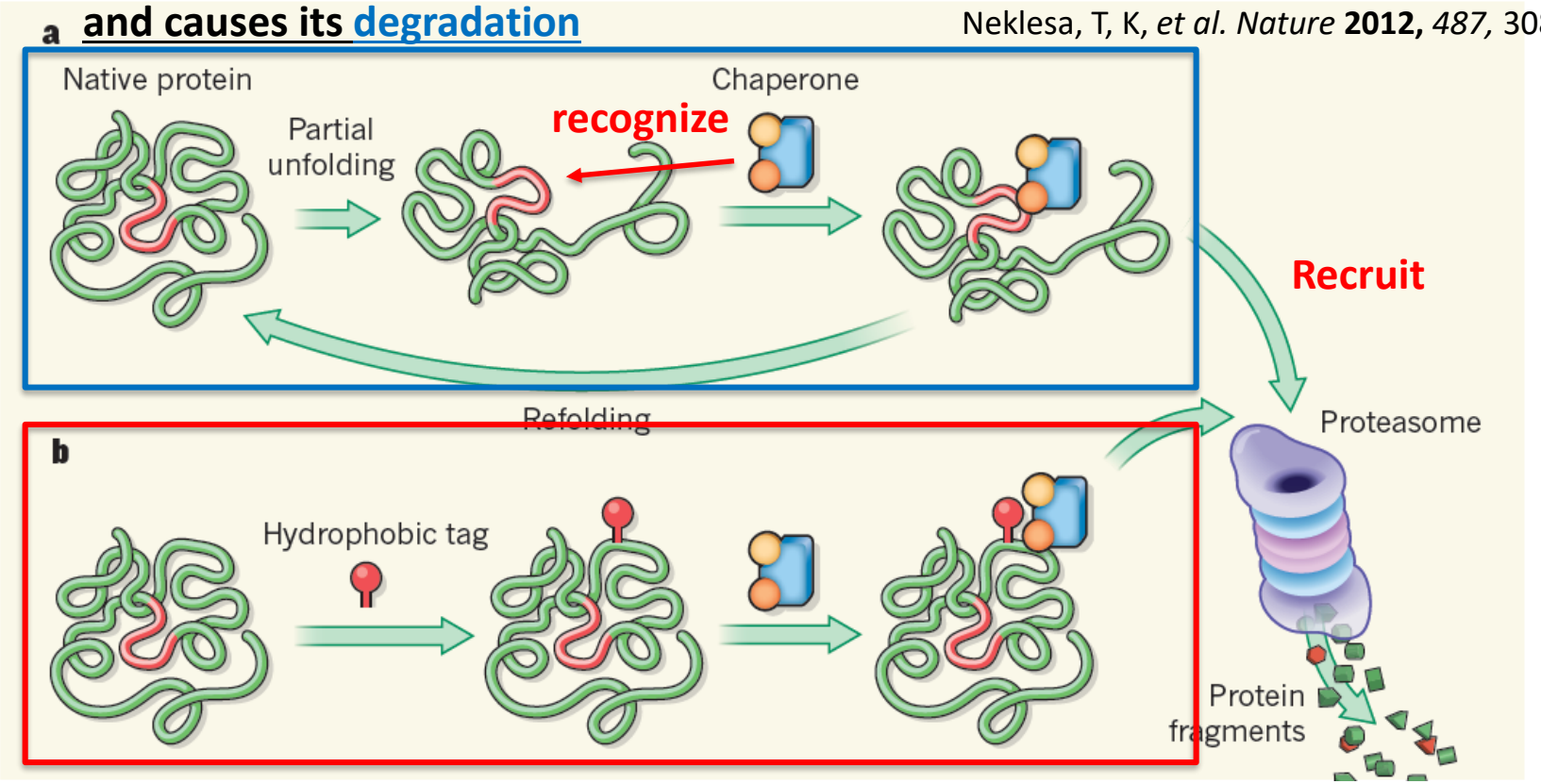
Dr. Kanemaki

Development of HyT system

Concept of Hydrophobic Tagging (HyT) technology

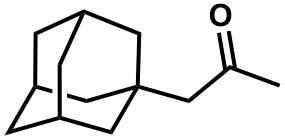
Chaperone recognizes exposed hydrophobic regions of unfolded protein and causes its degradation

Neklesa, T, K, *et al. Nature* **2012,** *487,* 308-9

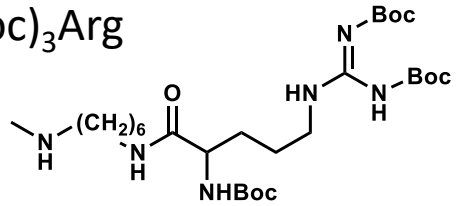


Hydrophobic tagging by small molecule may lead to protein degradation

Adamantyl group

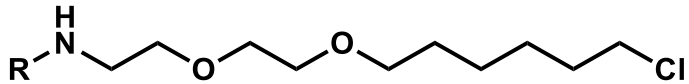


$(Boc)_3Arg$

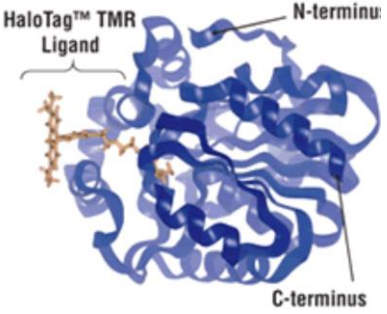


Development of HyT system

Hydrophobic tags destabilizing Halo Tag fusion proteins

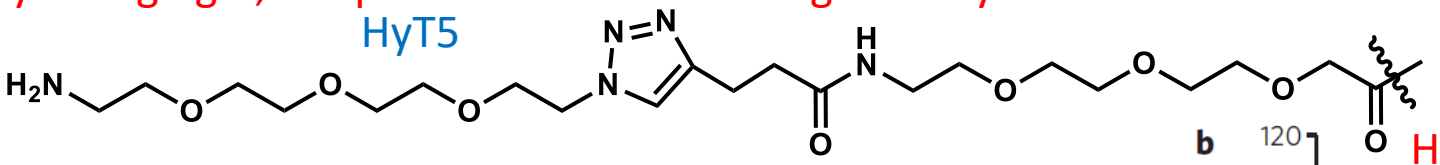


HaloTag reactive linker
(binds covalently to HaloTag)

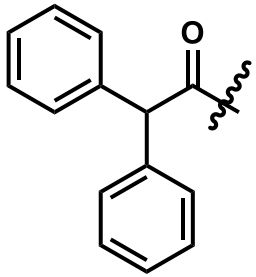


By changing R, the protein fused to HaloTag is easily studied

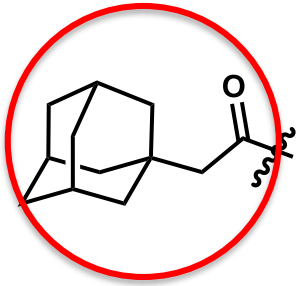
HyT5



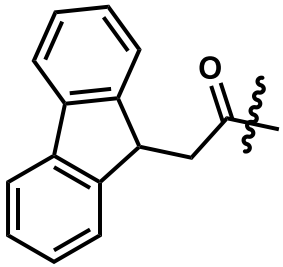
HyT12



HyT13

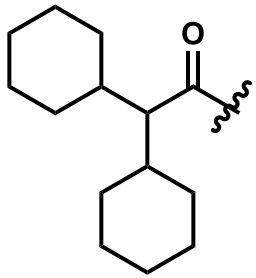


HyT16

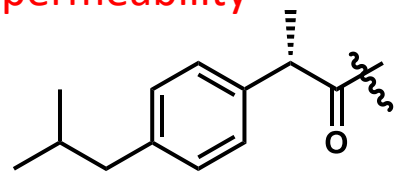


High stability
High cell permeability

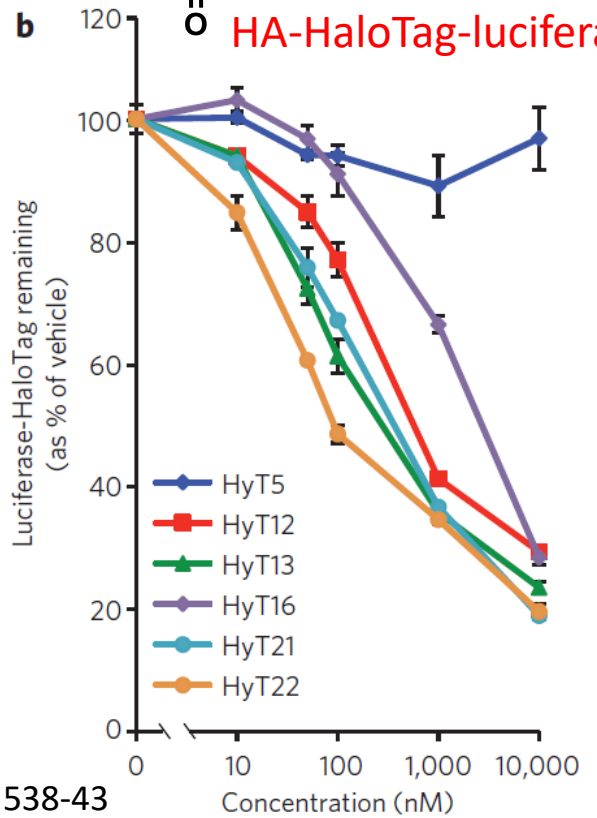
HyT21



HyT22

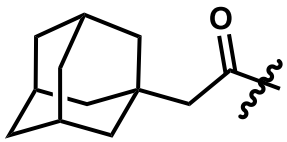


HA-HaloTag-luciferase

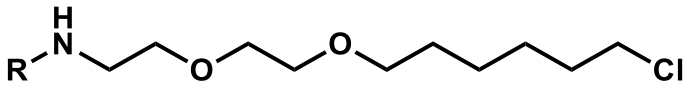


Neklesa, T, K, *et al. Nat. Chem. Biol* **2011,** *7,* 538-43

Development of HyT system



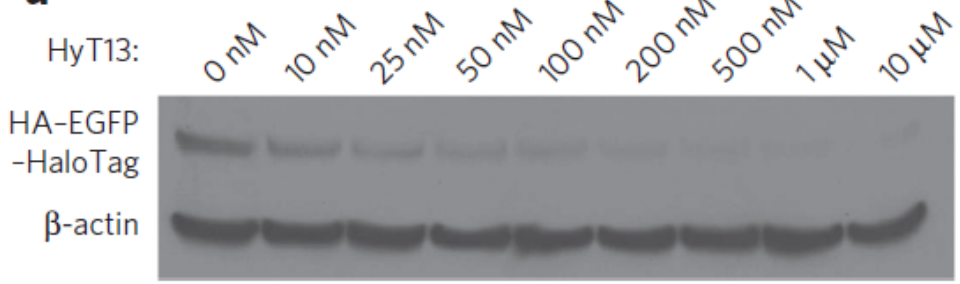
HyT13



HaloTag reactive linker
(binds covalently to HaloTag)

HyT13 destabilizes HA-EGFP-HaloTag

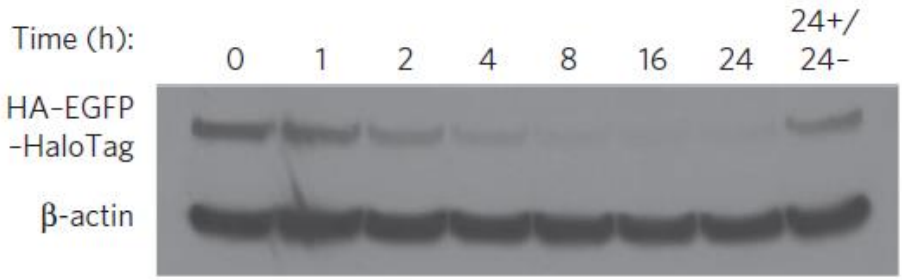
a 24h



Maximal degradation: 100nM

Time course of destabilization of HA-EGFP-HaloTag by HyT13

1uM

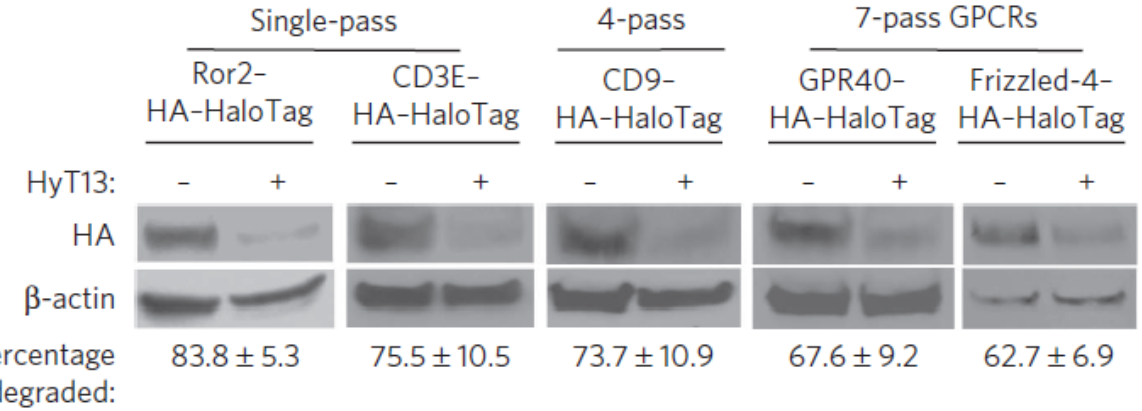


8h for full degradation, 1.5h for 50%

HyT13 can destabilize various proteins regardless of C- or N-terminus HaloTag is fused

1uM, 24h

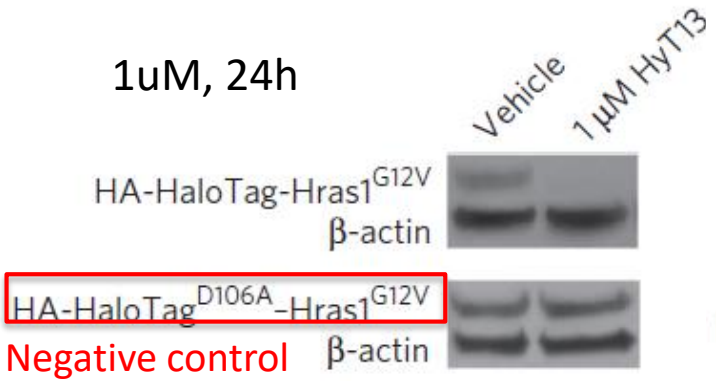
Neklesa, T, K, *et al. Nat. Chem. Biol* **2011,** *7,* 538-43



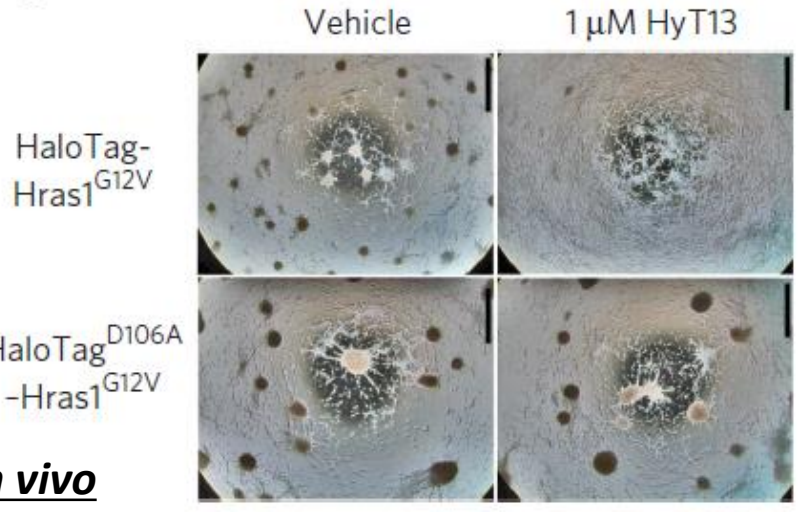
Development of HyT system

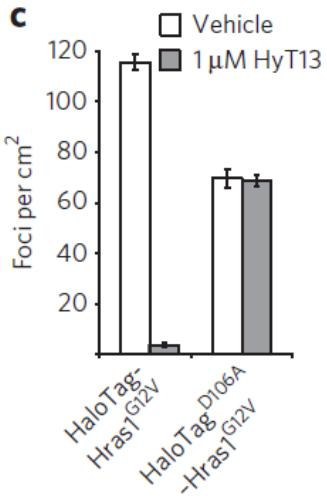
HyT13 suppresses HaloTag-HRas1(G12V)-driven tumor

HyT13 destabilizes HA-HaloTag-HRas1(G12V) *in cell*

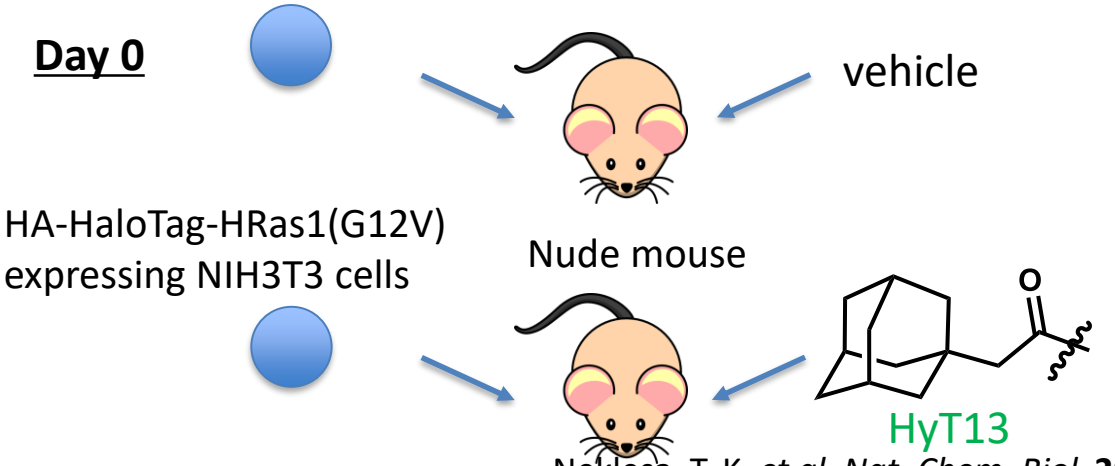


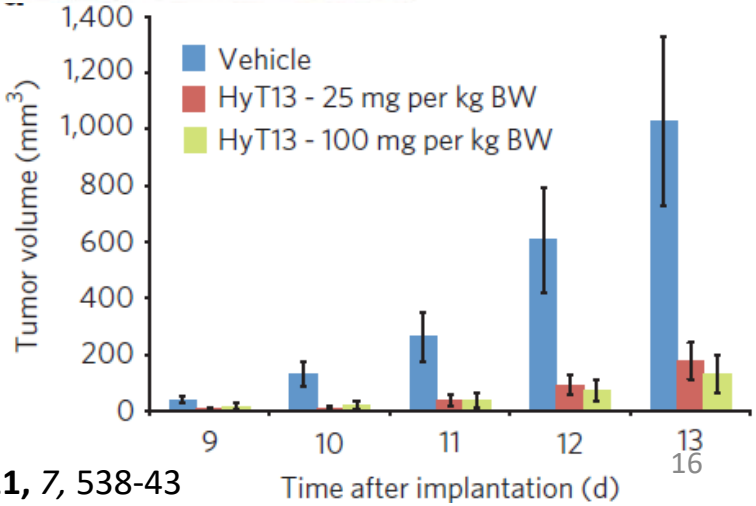
HyT13 suppresses the formation of foci, a marker of HRas1(G12V) tumor *in cell*





HyT13 suppresses the tumor growth *in vivo*



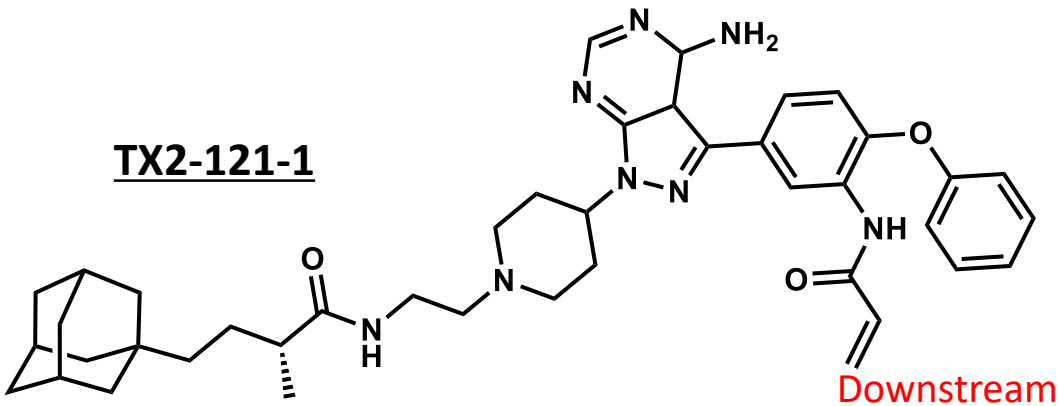


Neklesa, T, K, *et al. Nat. Chem. Biol* **2011,** *7,* 538-43

Development of HyT system

Her3 ligand-amantadine conjugate can target Her3

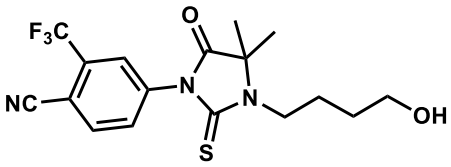
TX2-121-1



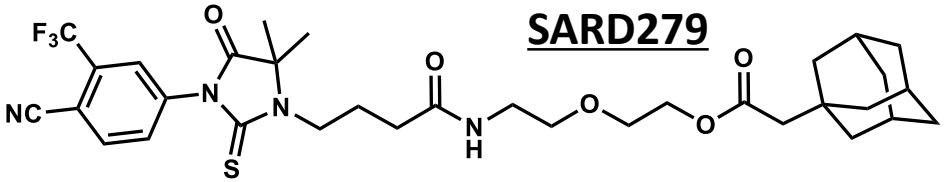
Xie, T, *et al. Nat. Chem. Biol* **2014,** *10,* 1006-12

HyT compound for Androgen receptor

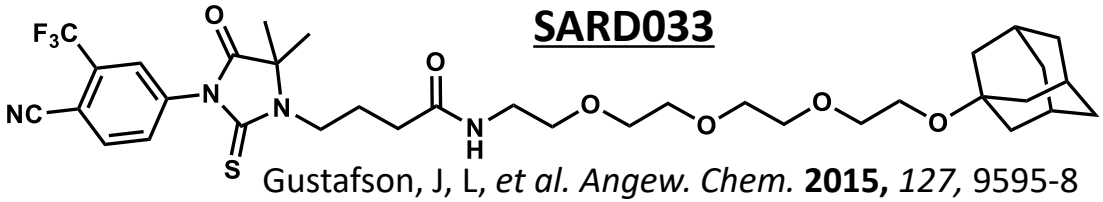
RU59063



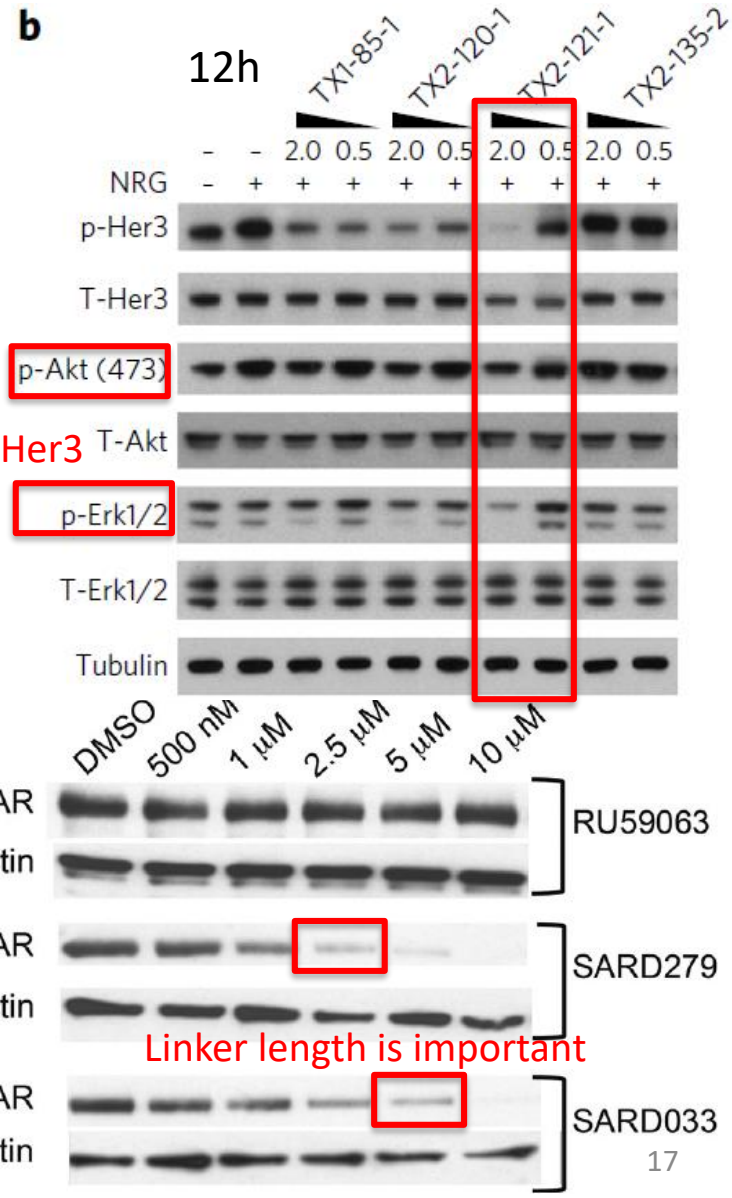
SARD279



SARD033



Gustafson, J, L, *et al. Angew. Chem.* **2015,** *127,* 9595-8



Short summary of HyT system

No Genetic manipulation

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peptide

2nd generation PROTACs

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Small-molecule

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2015 CRBN based

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2012 (Boc)3Arg

Small-molecule

Genetic manipulation

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DD system based

2006 DD and Shld-1

Small-molecule

✔temporal and dose-dependent

✔recovery of protein

✔*in vivo* application

×long and high dose

PROTAC is improved to small molecule induced technology without genetic manipulation

overview of 2nd generation PROTACs

No Genetic manipulation

1st generation PROTACs

2001 Protac-1

2004 Protac-4

peptide

Dr. Crews

2nd generation PROTACs

2008 MDM2 based

2011 cIAP-1 based

VHL based

2015 CRBN based

Small-molecule

HyT system

2011 Adamantane

2012 (Boc)3Arg

Small-molecule

Four types have been developed based on the kind of E3 ligase.

Potent ligand for E3 ligase is necessary.

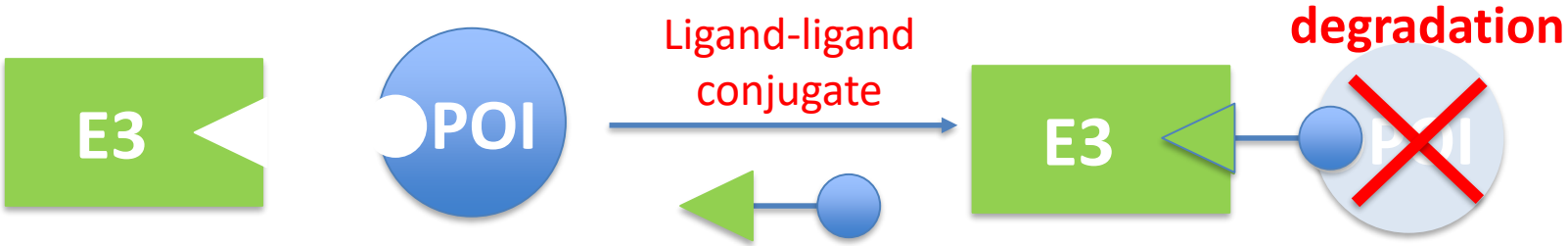
MDM2 or cIAP-1 based PROTACs were effective at >1uM order.

Selectivity among proteins in the same family as POI differed between VHL and CRBN based PROTACs.

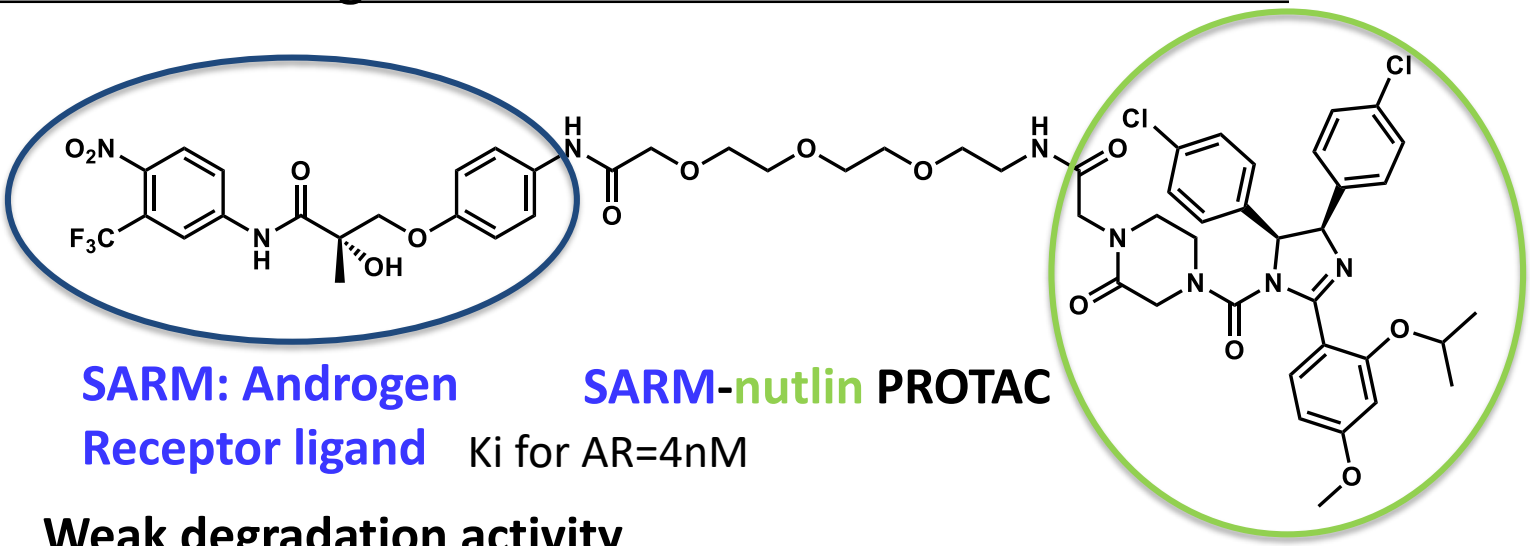
Linker length and position is important

VHL based PROTACs are mainly explained here.

First example of small-molecule PROTACs



Small molecule ligands were selected for both of E3 and POI

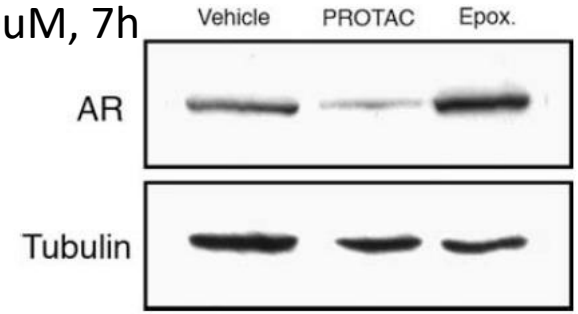


SARM: Androgen Receptor ligand Ki for AR=4nM

SARM-nutlin PROTAC

Weak degradation activity

10uM, 7h



IC50: 13.6uM or 0.09uM(enantiomer)

Nutlin-3: ligand for MDM2(E3 ligase)

Higher affinity ligands were sought after

Schneekloth, A. R., *et al. Bioorganic & Medicinal Chemistry Letters* **2008,** *18,* 5904-8

improved small-molecule PROTACs (VHL based)

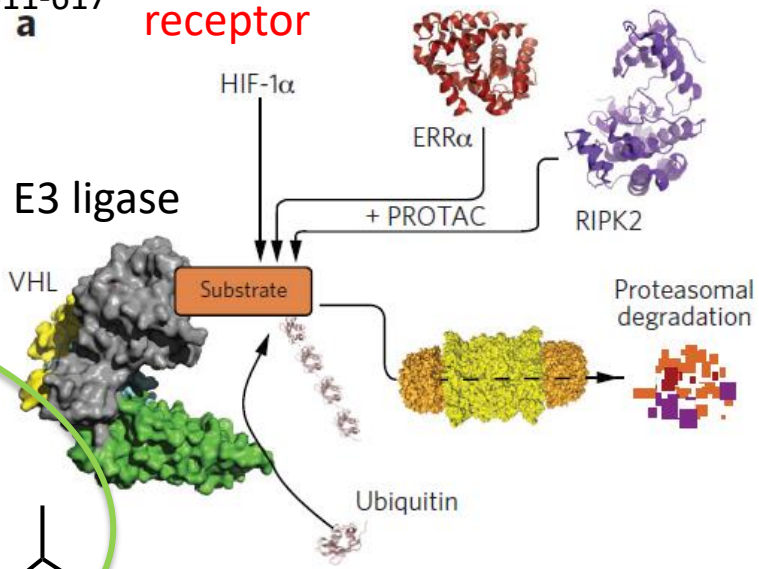
PROTACs targeting ERRα and RIPK2 were designed

Bondeson, D, P, *et al. Nat. Chem. Biol* **2015,** *11,* 611-617

PROTAC_ERRα

Comp. 29: binds to ERRα

ERRα: estrogen related receptor



RIPK2: serine/ threonine kinase

VHL ligand

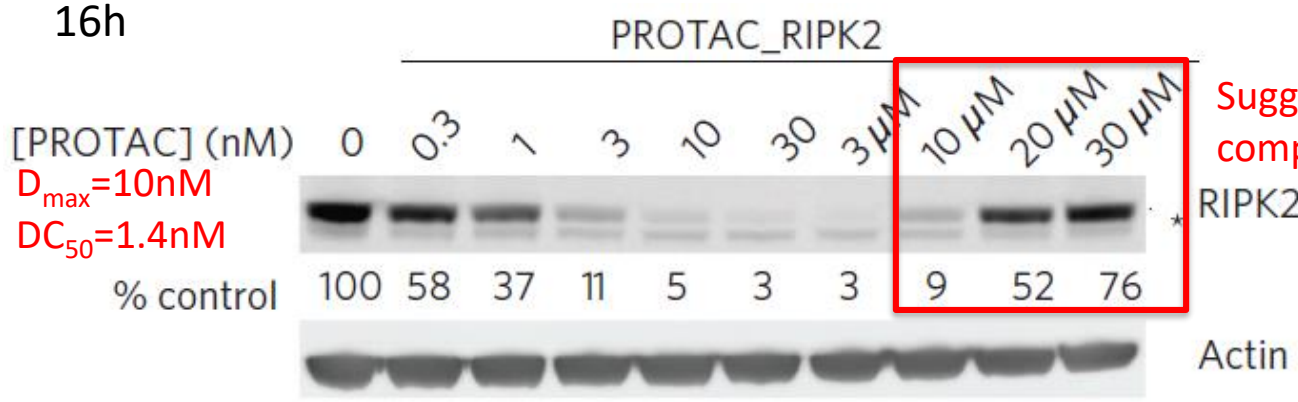
K_d=320nM

Vandetanib: tyrosine kinase inhibitor

PROTAC_RIPK2

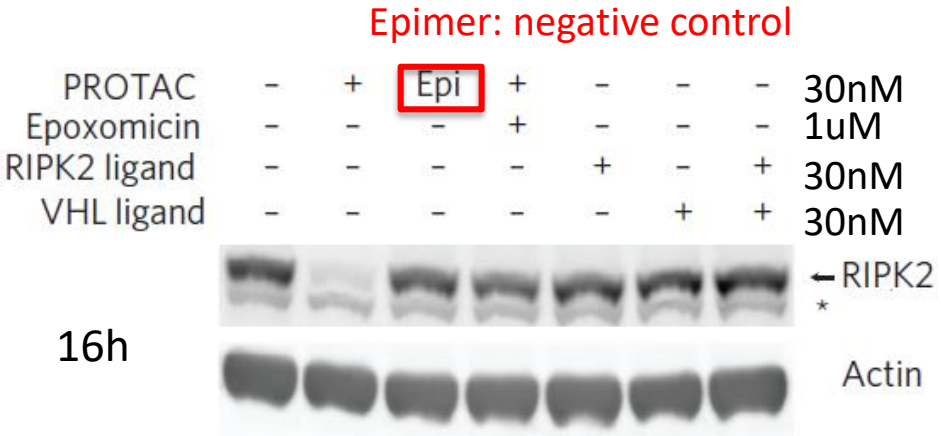
improved small-molecule PROTACs (VHL based)

PROTAC_RIPK2 can dose-dependently downregulate RIPK2

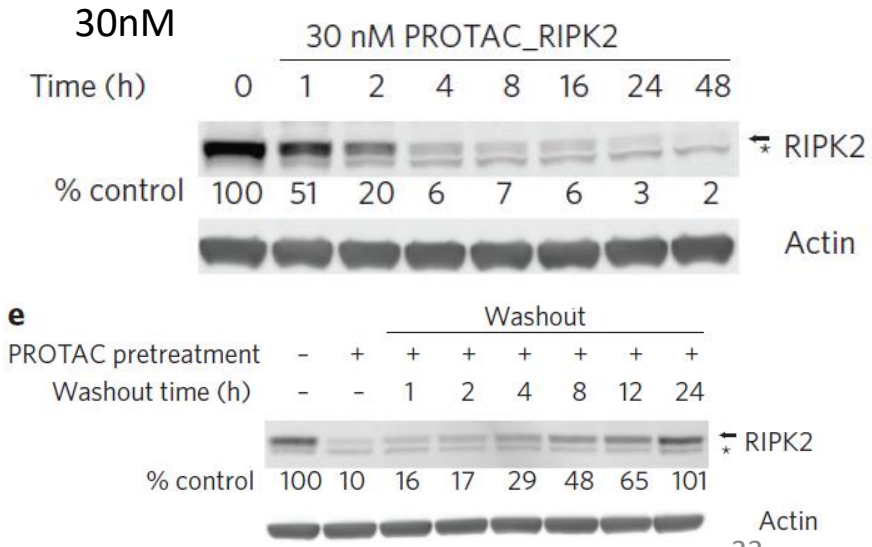


Suggests the need of ternary complex formation

Ternary complex formation and proteasome is necessary for downregulation of RIPK2



Time-course of degradation and recovery



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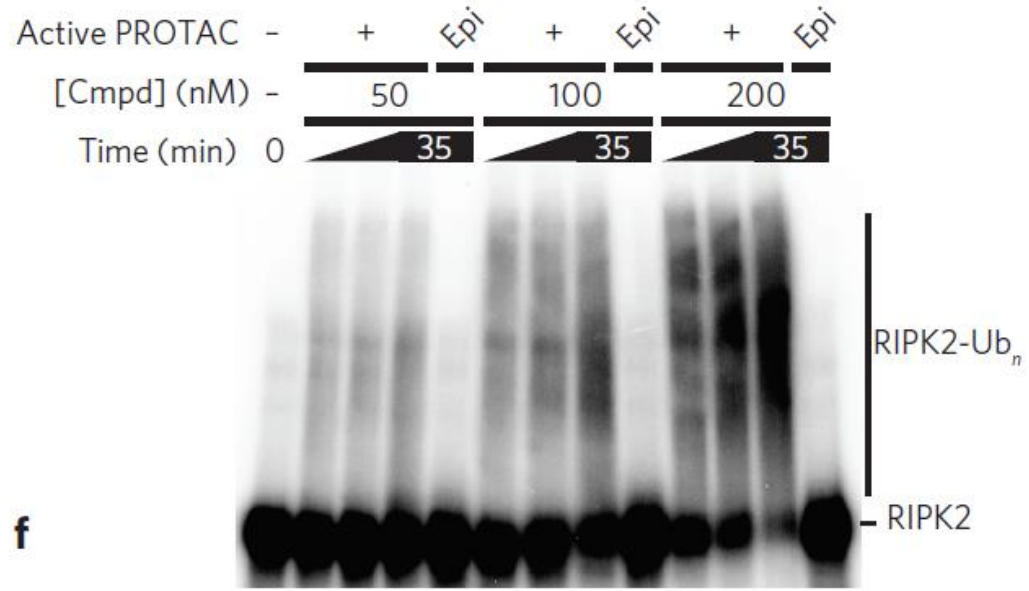
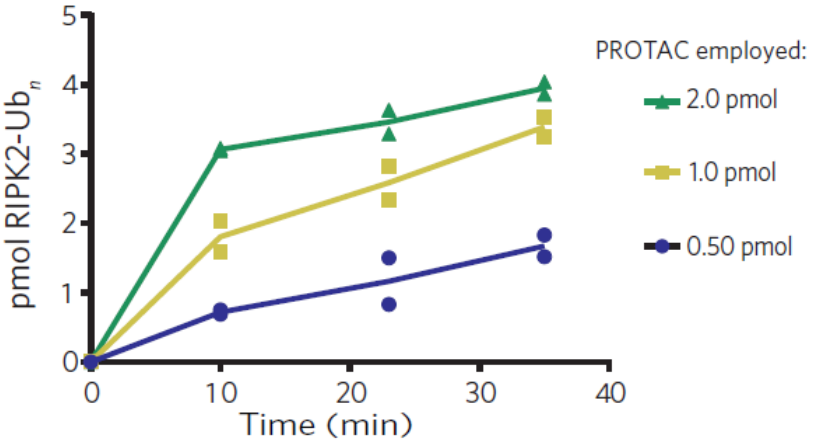
improved small-molecule PROTACs (VHL based)

PROTAC_RIPK2 induces ubiquitination of RIPK2 dose-dependently and time-dependently



Catalytic nature of PROTAC_RIPK2



PROTAC (pmol):	0.5	1.0	2.0
RIPK2-Ub (pmol):	1.7	3.4	4.0
Stoichiometry:	3.3	3.4	2.0

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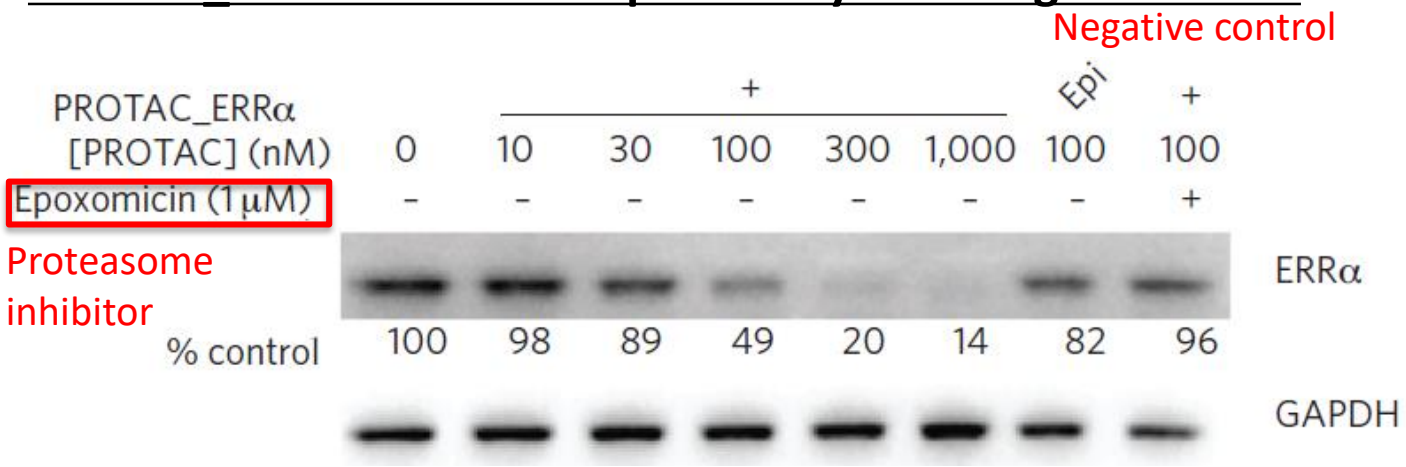
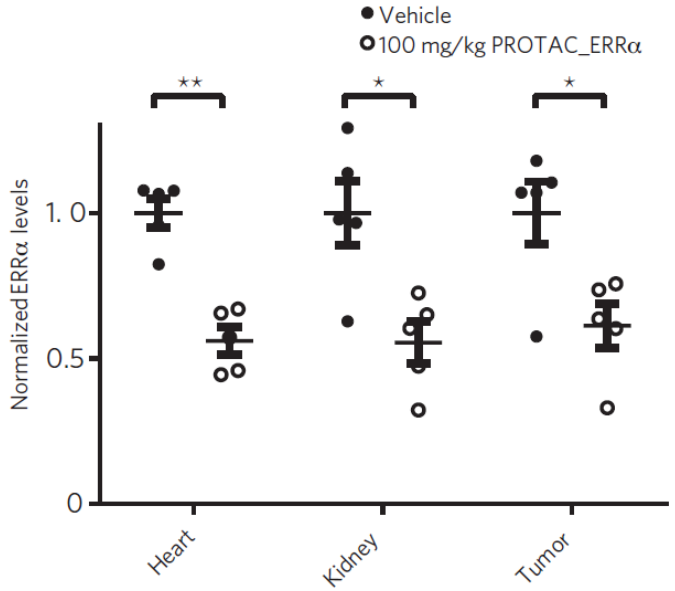
improved small-molecule PROTACs (VHL based)

PROTAC_ERRα can dose-dependently downregulate ERRα



PROTAC_ERRα can target ERRα *in vivo*

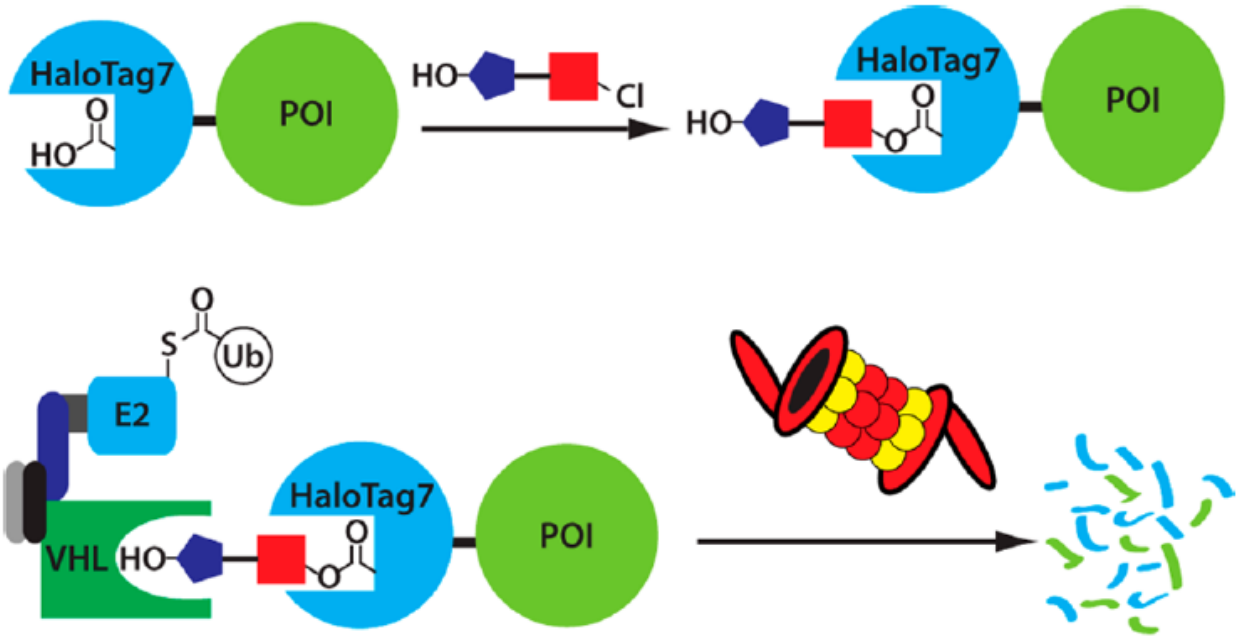


Bondeson, D, P, *et al. Nat. Chem. Biol* **2015,** *11,* 611-617

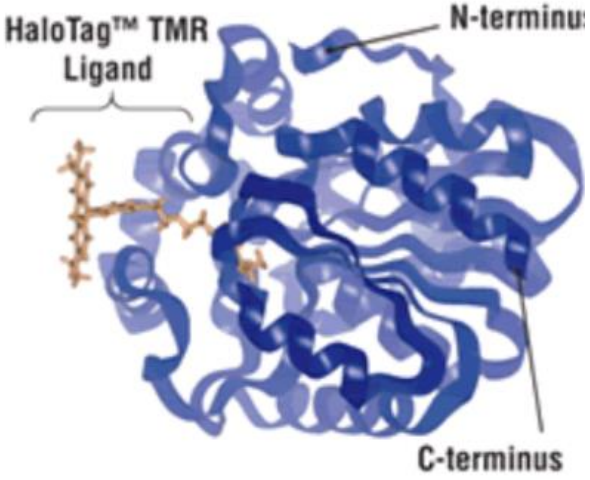
HaloPROTACs (VHL based)

HaloTag can expand the variety of POI

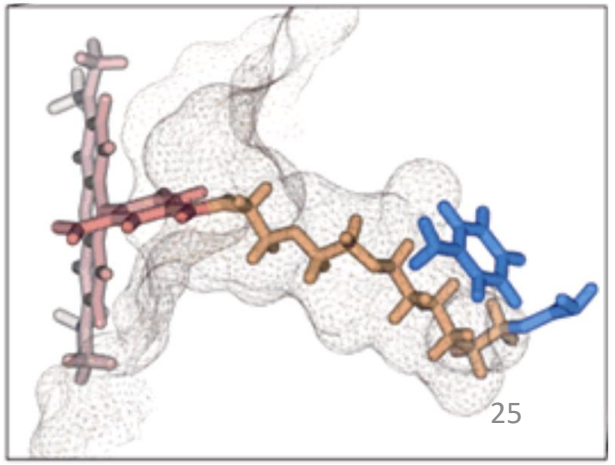
Buckley, D, L, *et al. ACS Chem. Biol.* **2015,** *10,* 1831-7



HaloTag structure with ligand



Close-up



HaloPROTACs (VHL based)

Linker position and length are important for HaloPROTACs' activity

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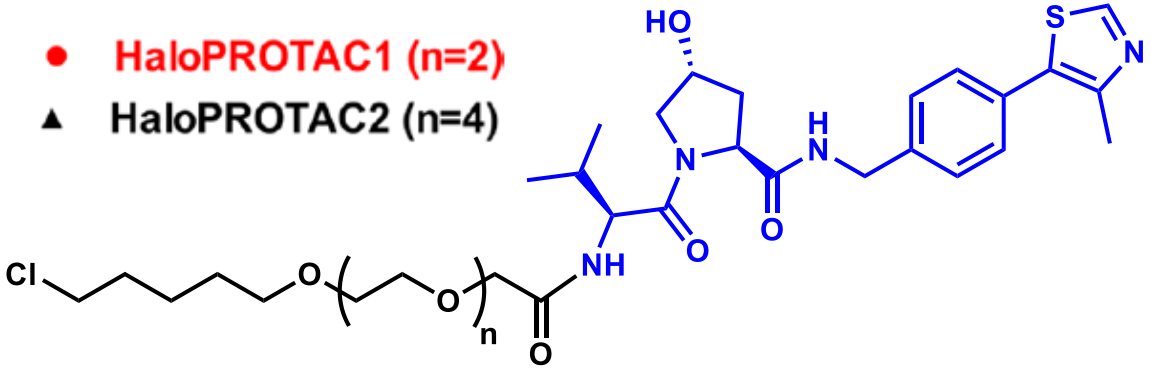
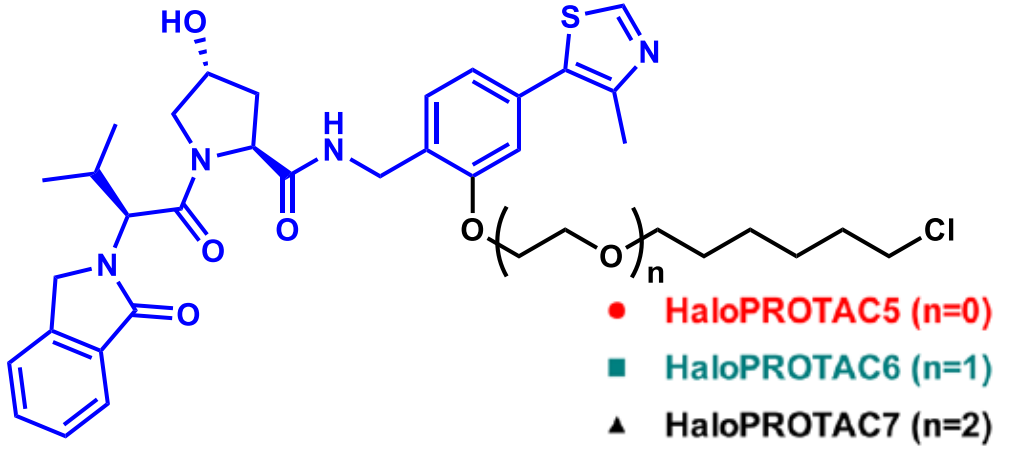
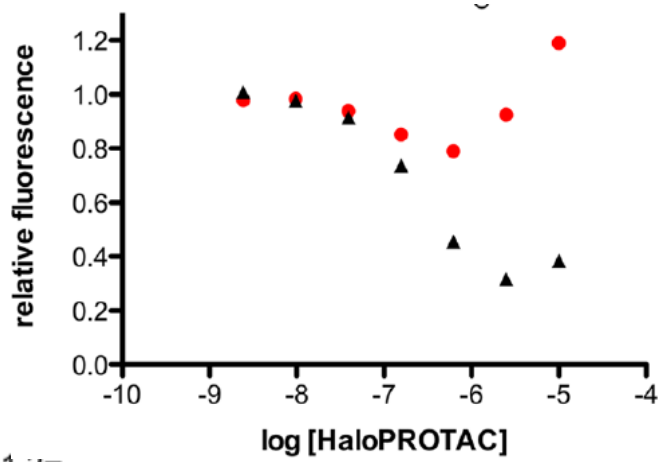
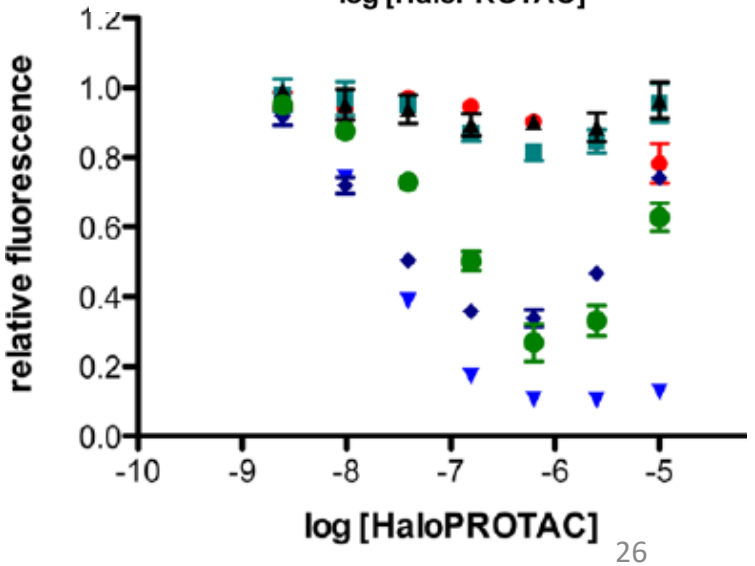
● HaloPROTAC1 (n=2)
▲ HaloPROTAC2 (n=4)





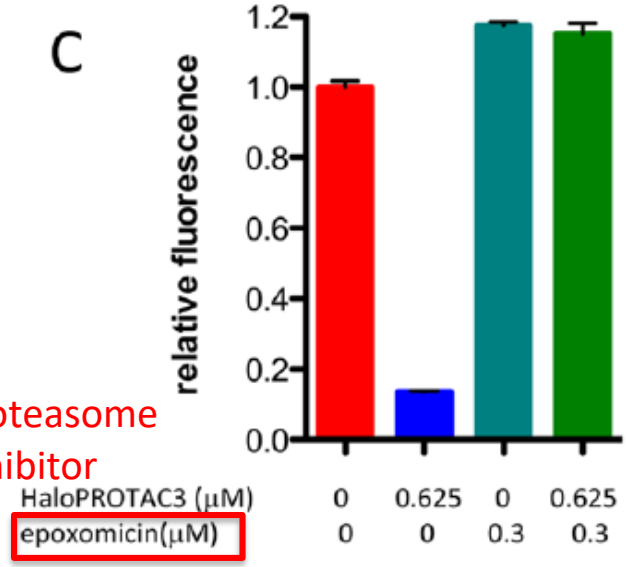
● HaloPROTAC5 (n=0)
■ HaloPROTAC6 (n=1)
▲ HaloPROTAC7 (n=2)
best ▼ HaloPROTAC3 (n=3)
◆ HaloPROTAC8 (n=4)
● HaloPROTAC4 (n=5)





HaloPROTACs (VHL based)

Proteasome inhibitor suppresses the degradation caused by HaloPROTAC3



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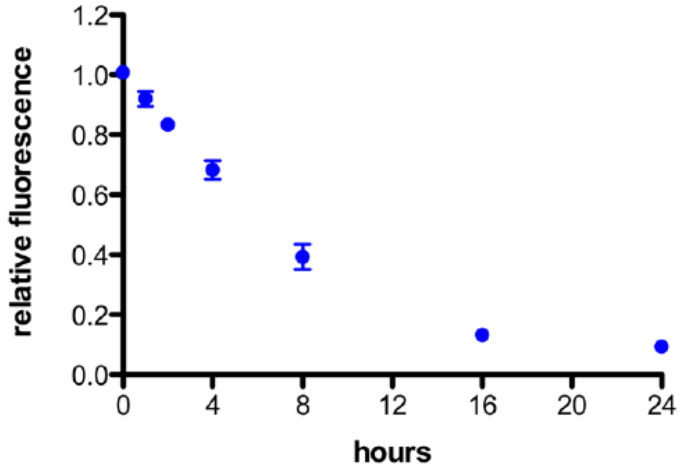
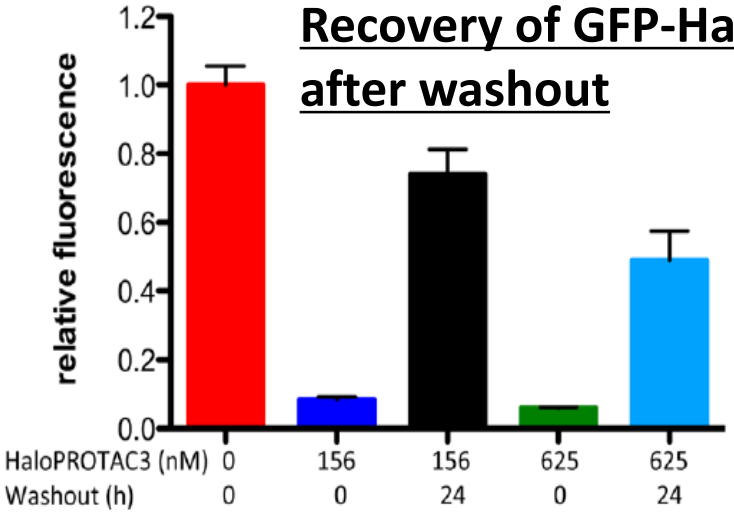
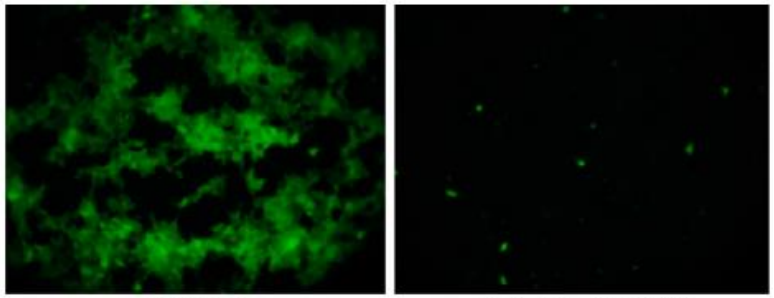
Time-course of GFP-HaloTag7 degradation



Recovery of GFP-HaloTag after washout

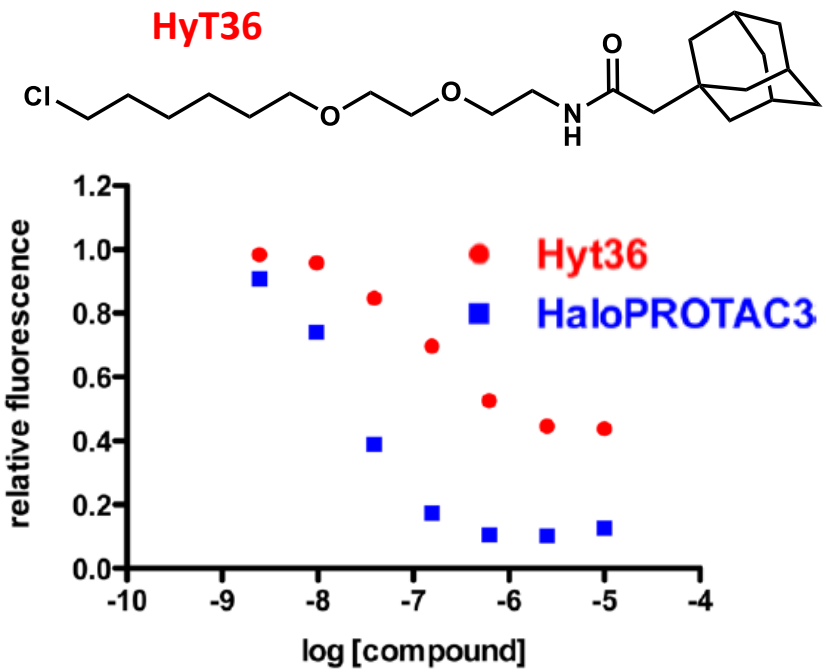


Microscopic data

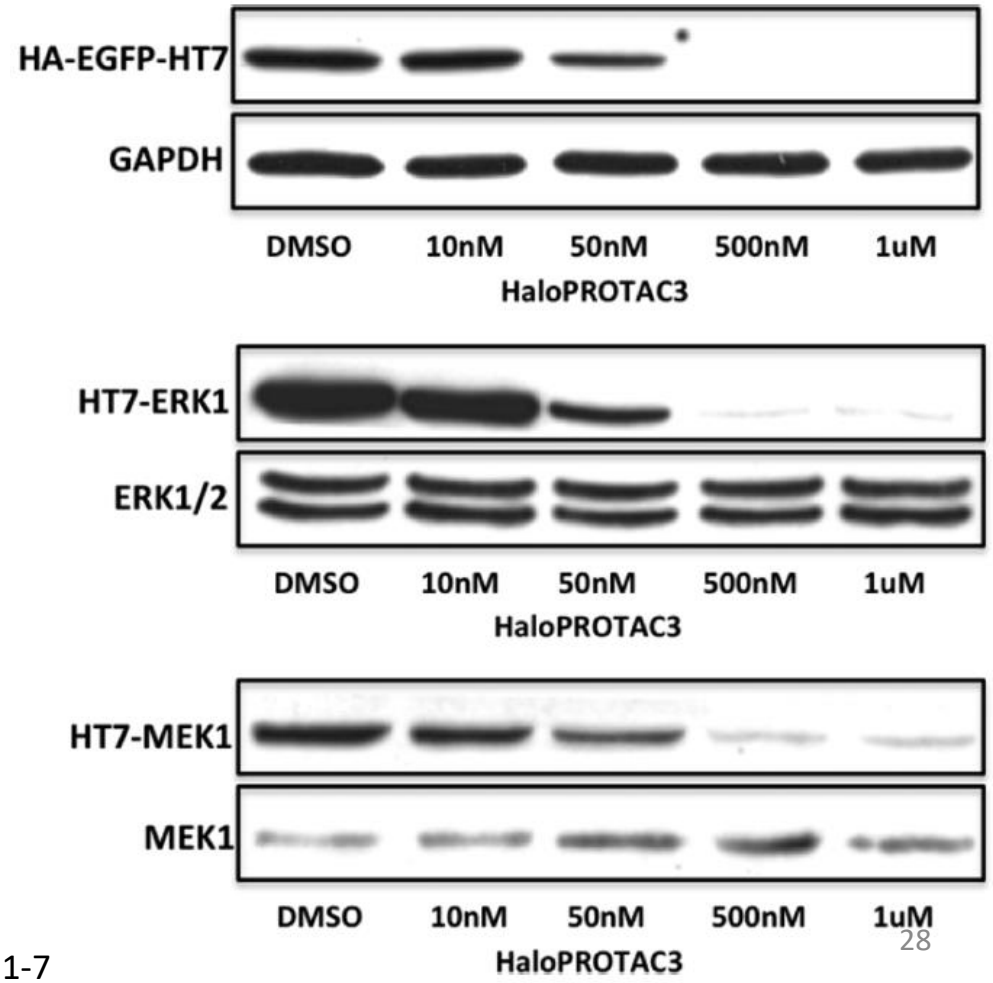


HaloPROTACs (VHL based)

HaloPROTAC3 can degrade GFP-HaloTag7 faster and more



HaloPROTAC3 can degrade other fusion proteins with HaloTag7



Buckley, D, L, *et al. ACS Chem. Biol.* **2015,** *10,* 1831-7

Short summary of small-molecule PROTACs

No Genetic manipulation

1st generation PROTACs

Dr. Crews

2001 Protac-1

2004 Protac-4

peptide

2nd generation PROTACs

2008 MDM2based

Small-molecule

2011 cIAP-1 based

VHL based

2015 CRBN based

HyT system

2011 Adamantane

2012 (Boc)3Arg

Small-molecule

Genetic manipulation

Dr. Wandless

✔temporal and dose-dependent

✔recovery of protein

✔catalytic

✔application to various proteins(HaloPROTACs)

✔*in vivo* application

×ligand dependent

Dr. Kanemaki

Summary

・conditional protein degradation is powerful in context of both biological research and drug discovery

Peptidic PROTACs

✔proof of concept

×difficulty in practical use

DD and Shld1 based systems/AID

✔high specificity due to genetic manipulation

×limited application due to genetic manipulation

HyT

✔in vivo application, low toxicity

×relatively high dose

Small-molecule PROTACs

✔in vivo application, quite low dose

・room for optimization

Tools to control protein degradation conditionally

No Genetic manipulation

1st generation PROTACs

Dr. Crews

2001 Protac-1

peptide

2004 Protac-4

2nd generation PROTACs

2008 MDM2based Small-molecule

HyT system

2011 cIAP-1 based 2011 Adamantane

VHL based 2012 $(Boc)_3Arg$

2015 CRBN based

Small-molecule

Genetic manipulation

Dr. Wandless

DD system based

2006 DD and Shld-1

Small-molecule

AID system

2009 AID system

Small-molecule

2011 LID system

Small-molecule

2013 B-LID system

Blue light

Dr. Kanemaki