Targeted Protein Degradation as Emerging Antiviral Therapeutics

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16/12/2021
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Outline

PART 01 Protein degradation

PART 02 Targeted protein degradation(TPD)

- Principal
- Advantages
- Applications

PART 03 TPD as antiviral therapeutics

- Anti-HBV
- Anti-HCV
- Anti-Cov

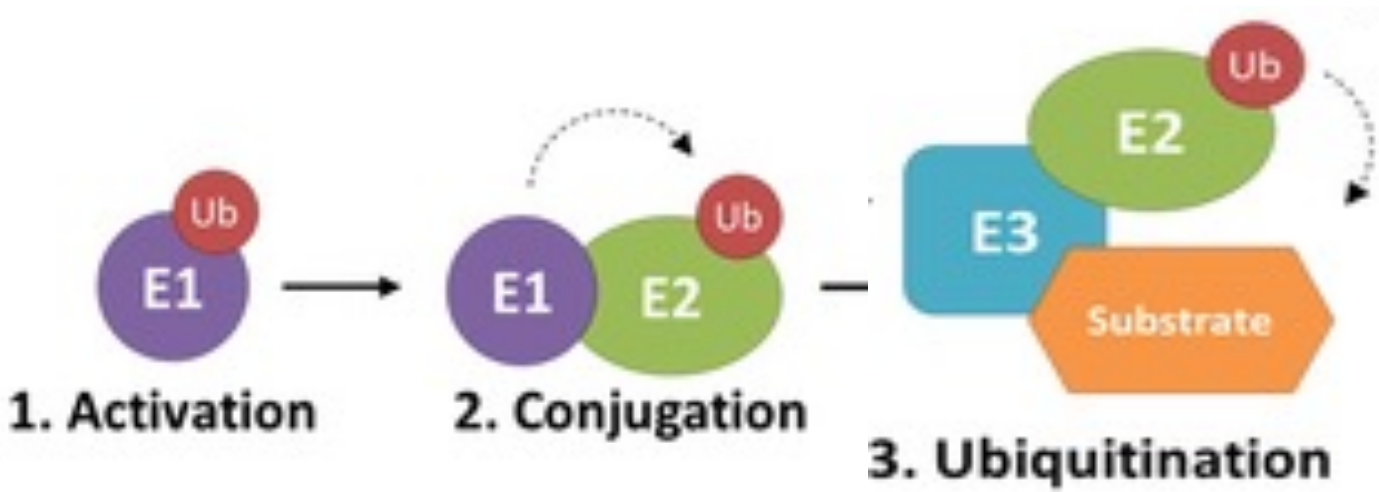
PART 04 Challenges of TPD

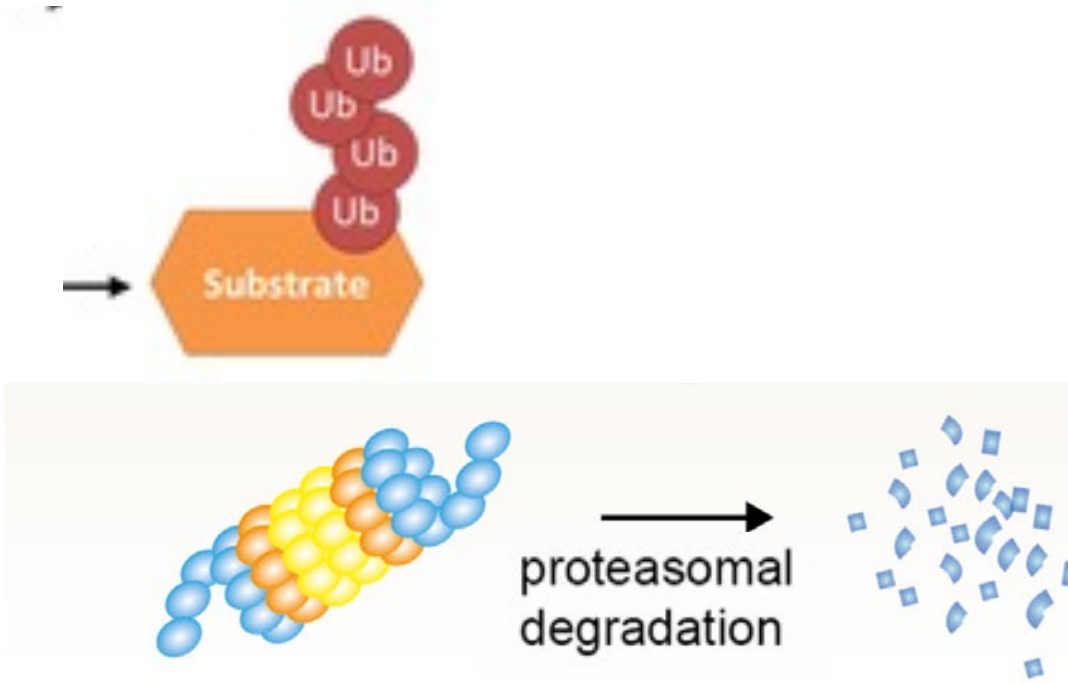
Protein degradation

- Misfolded proteins
- Maintaining protein homeostasis

Ubiquitin-proteasome System (UPS)

- 26S proteasome mediated





26S proteasome

Yang Wang, et al, Acta Pharmaceutica Sinica B 2020;

Protein degradation

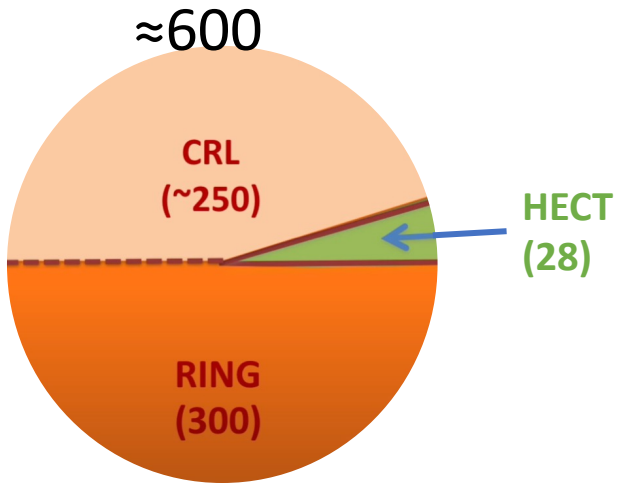
E3-ubiquitin ligase

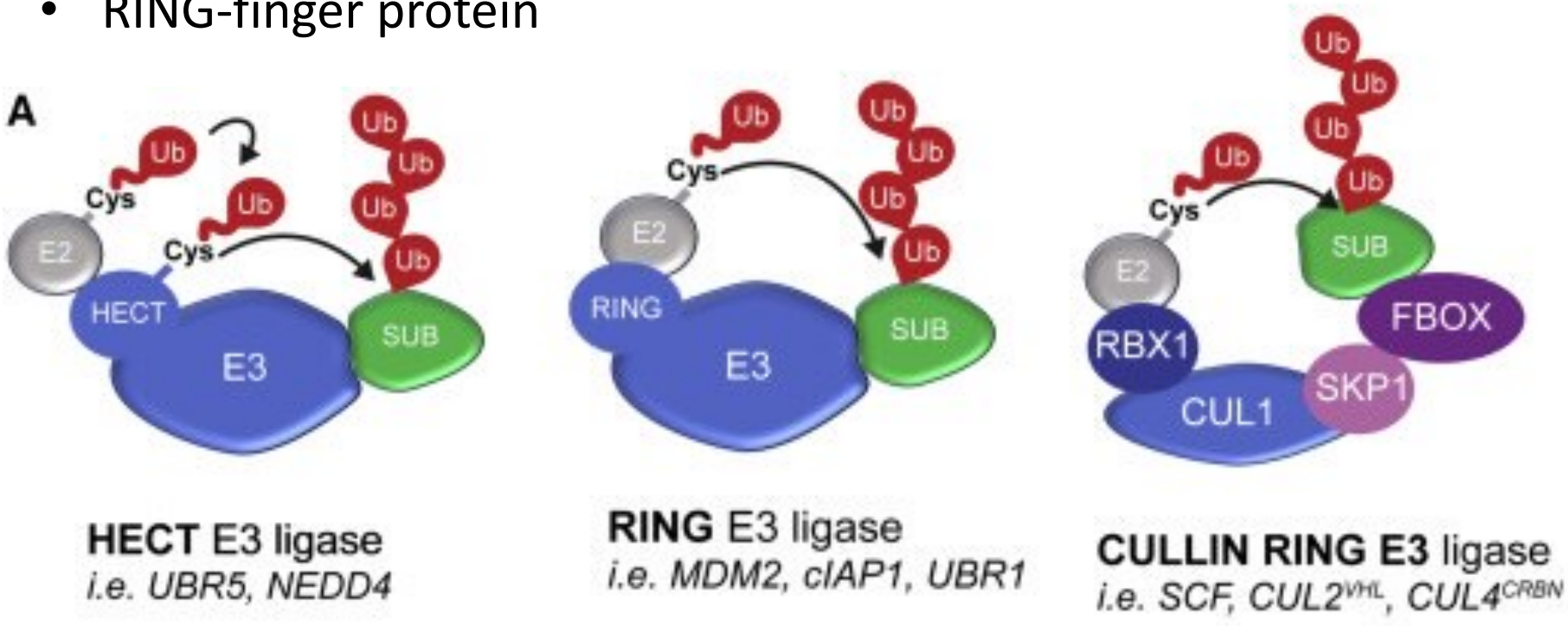
(N)Target recognizing domain

- Specific recognition
- Specificity and versatility

(C)E2 interacting domain

- HECT
- RING-finger protein





HECT E3 ligase
i.e. UBR5, NEDD4

RING E3 ligase
i.e. MDM2, cIAP1, UBR1

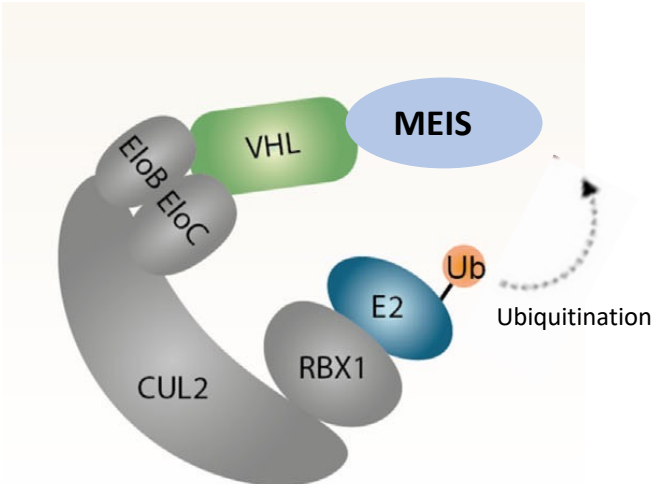
CULLIN RING E3 ligase
i.e. SCF, CUL2^{VHL}, CUL4^{CRBN}

Predrag Jevtić, Cell Chemical Biology,2021

Principal of Targeted Protein Degradation(TPD)

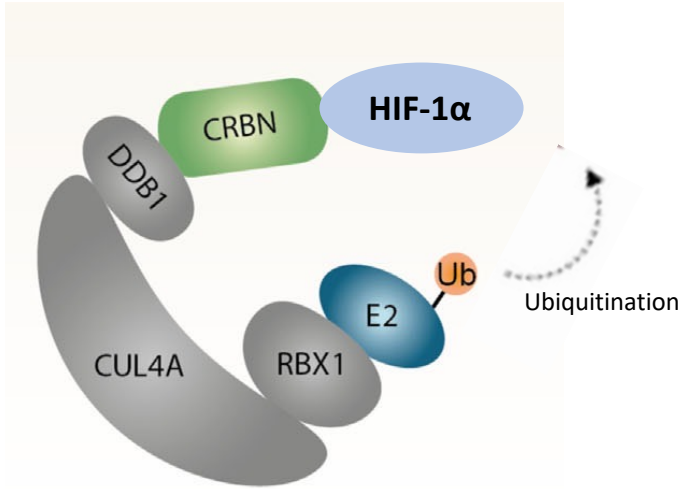
PROTAC

- PROteolysis TArgeting Chimera
- Synthesized hetero bi-functional degrader



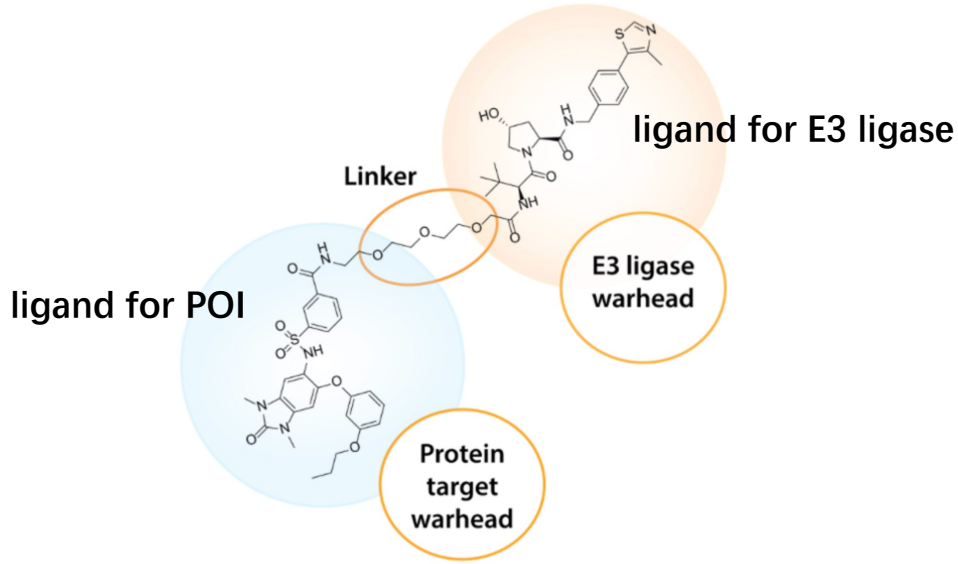
$CUL2^{VHL}$

VHL(von Hippel–Lindau disease tumor suppressor)



$CUL4^{CRBN}$

CRBN (Cereblon)



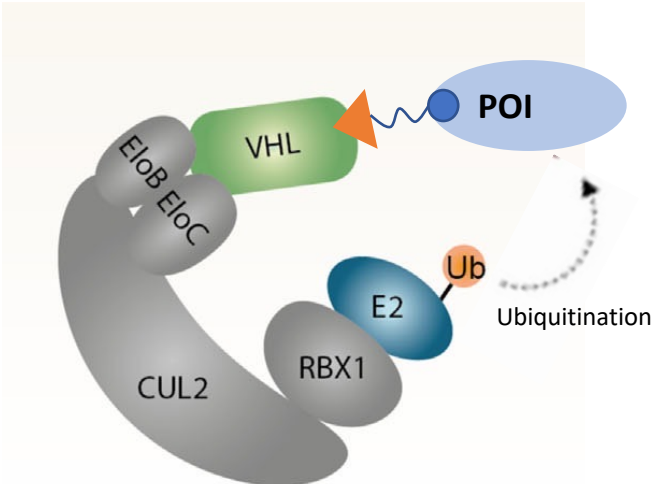
Bondeson DP, et al. Nat Chem Biol. 2015

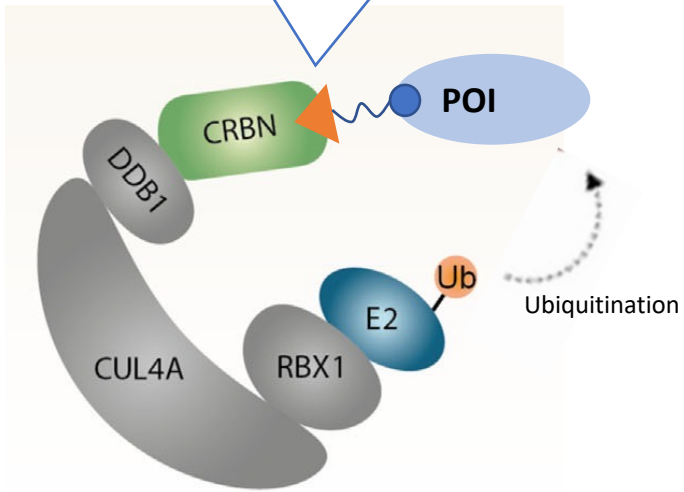
Laura M. Luh et al, Angewandte Chemie International Edition · 2020

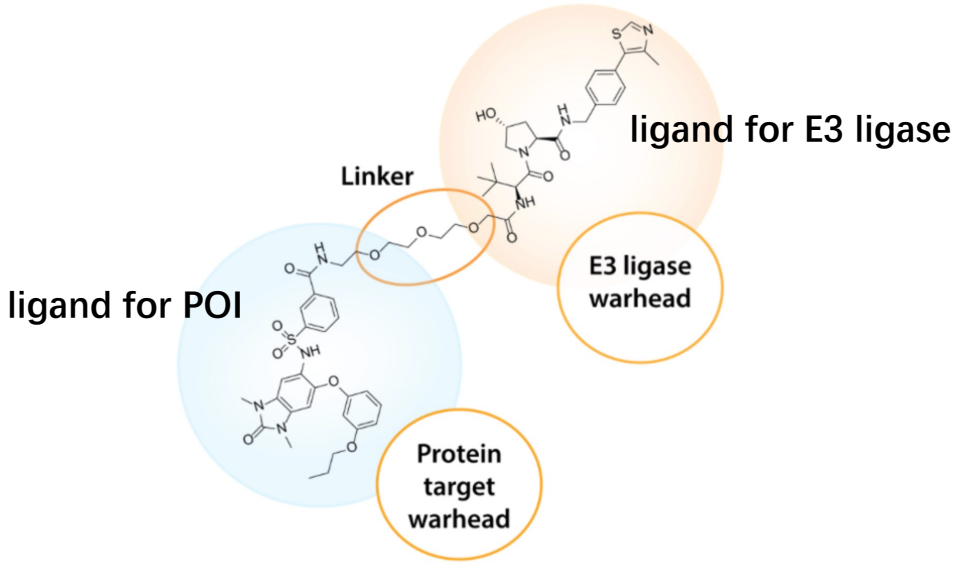
Principal of Targeted Protein Degradation(TPD)

PROTAC
- PROteolysis TArgeting Chimera
- Synthesized hetero bi-functional degrader

IMiDs
- Immunomodulatory imide drugs
- Thalidomide
- Analogues







$CUL2^{VHL}$

VHL(von Hippel–Lindau disease tumor suppressor)

$CUL4^{CRBN}$

CRBN (Cereblon)

Bondeson DP, et al. Nat Chem Biol. 2015

Laura M. Luh et al, Angewandte Chemie International Edition · 2020

Principal of TPD

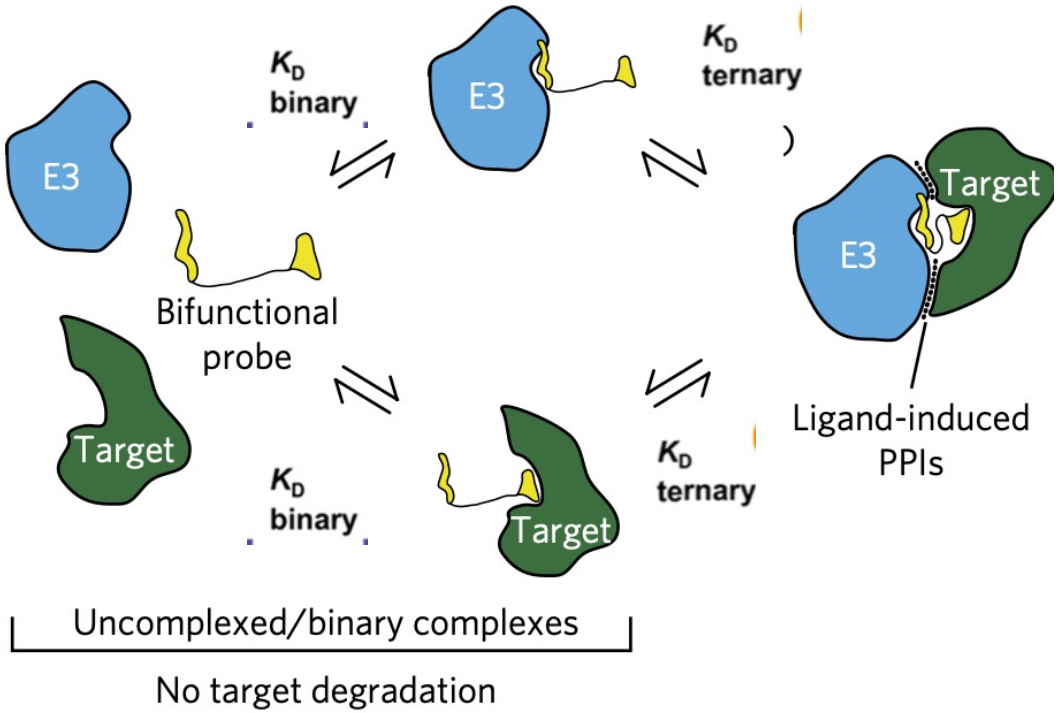
K_D
- dissociation constant
- describe binding affinity

($K_D = k_{off}/ k_{on}$)

cooperativity (α) factor
($\alpha = K_{D\ binary}/K_{D\ ternary}$)

- Co-IP, NanoBRET, Cell-free proximity assays (AlphaLISA, AlphaScreen), ITC DSF, FP, SPR, NMR

$t_{1/2}$
dissociative half-life of the ternary complex
($t_{1/2} = \ln 2/k_{off}$)

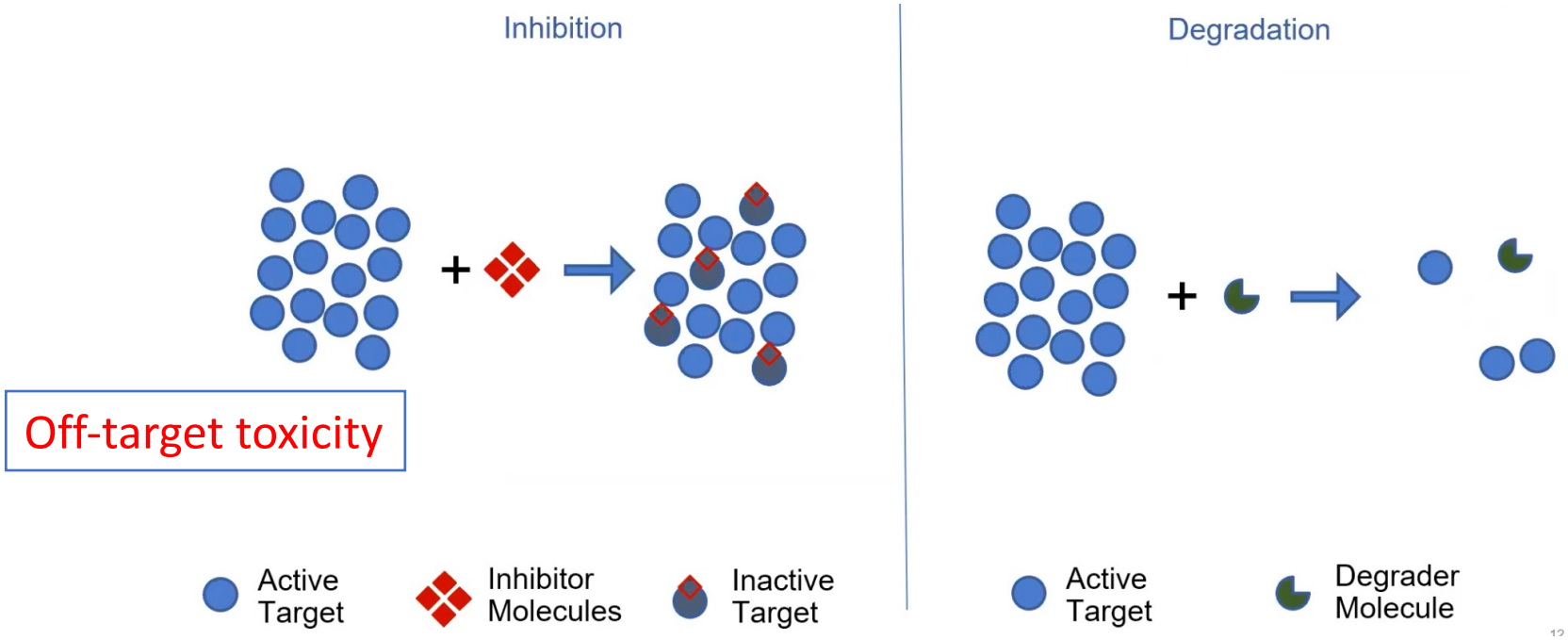


- Positive cooperativity ($\alpha > 1$)
- Highly populated
- Effective and selective target degradation

Scott J. Hughes , Essays in Biochemistry (2017)

Advantages of TPD

Kinase



Occupancy-driven

- Longer a drug blocks an active site
- Greater the clinical effect achieved
- Irreversible covalent

Event-driven

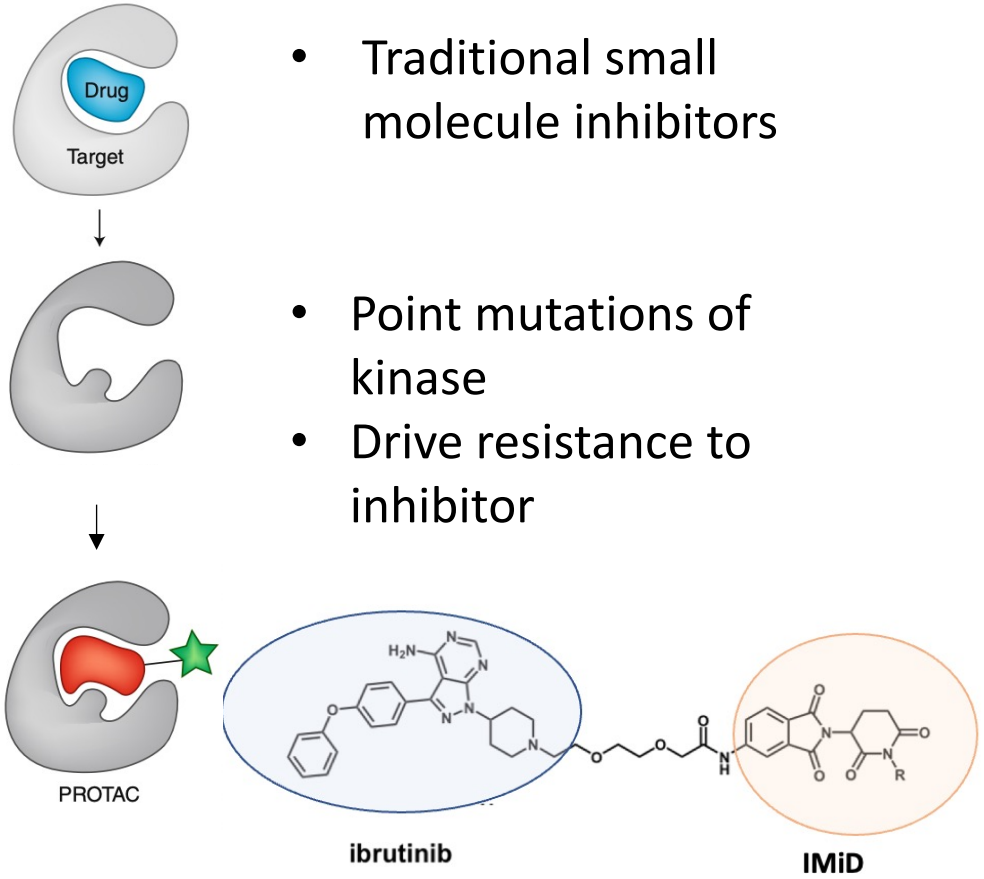
- Formation of the ternary complex
- Lower dose for potent degradation
- capable of complete removal of both the kinase and scaffolding functions

Philipp M CrommCel et al, Chemical Biology,2021

Advantages of TPD

Kinase

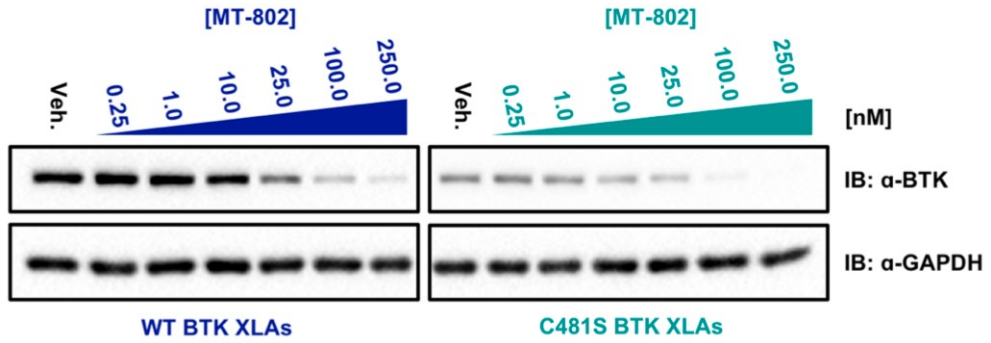
Overcome resistance



- Traditional small molecule inhibitors
- Point mutations of kinase
- Drive resistance to inhibitor

C481S BTK

- Substitution of the active-site cysteine residue to a serine
- Reduces binding of the covalent inhibitor ibrutinib
- Poorly inhibited
- Ibrutinib scaffold could still serve the role of a PROTAC warhead



PROTACs

- Based on the ibrutinib
- Reversible
- Help overcome resistance associated with mutations

Buhimschi, A. D. et al.. Biochemistry ,2018.

Advantages of TPD

Drugging the 'undruggable

'Undruggable', or 'challenging to drug'

- Cannot be always modulated by conventional small molecule inhibitors
- Transcription factors, scaffolding proteins
  - have catalytic independent functions

STAT3

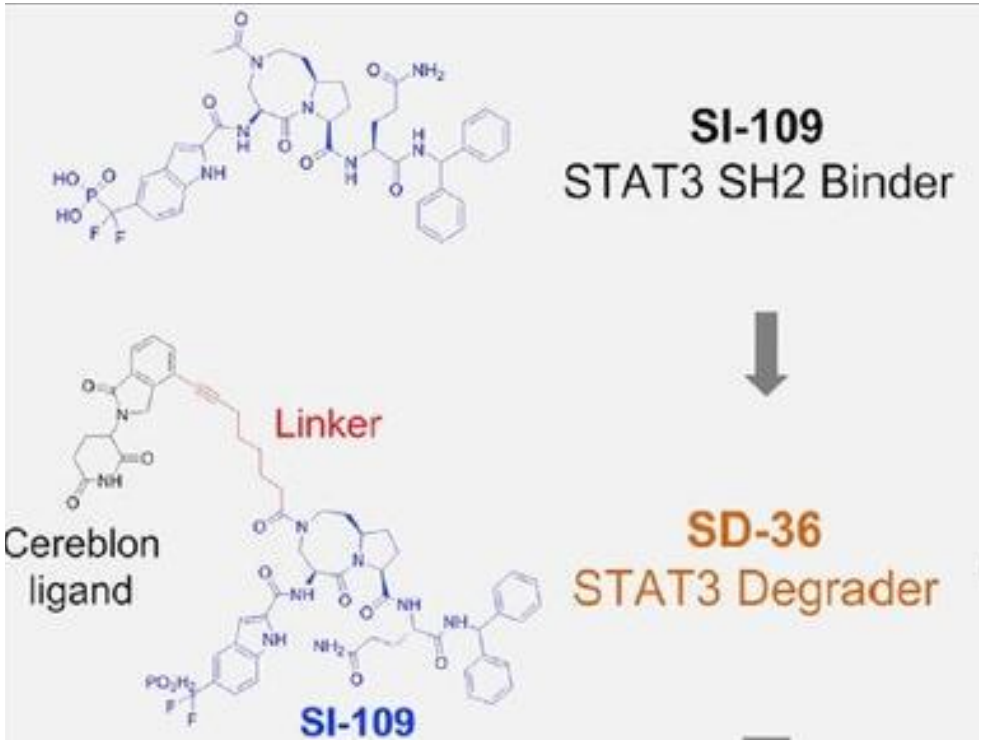
- Transcriptional regulator
- Linked to numerous cancers and other inflammatory diseases

Difficulty to obtain highly selective STAT3 inhibitors

1.STAT family members share a highly structurally homologous SH2 domain

2.Monomeric STAT3 protein also has transcriptional activity

an alternative approach



SD-36

- Achieved efficient and sustained degradation of STAT3
- Superior in a xenograft mouse model

Bai et al., Cancer Cell, 2019

Application of TPD

Clinical examples

ARV-471

- Significantly reduce ER expression level in tumor tissues, with an average of 62% and a maximum of 90%
- Both wild-type ER and mutant ER.

ARV-110

- Exhibited satisfactory safety and tolerability in patients
- High potency against both wild-type and mutants

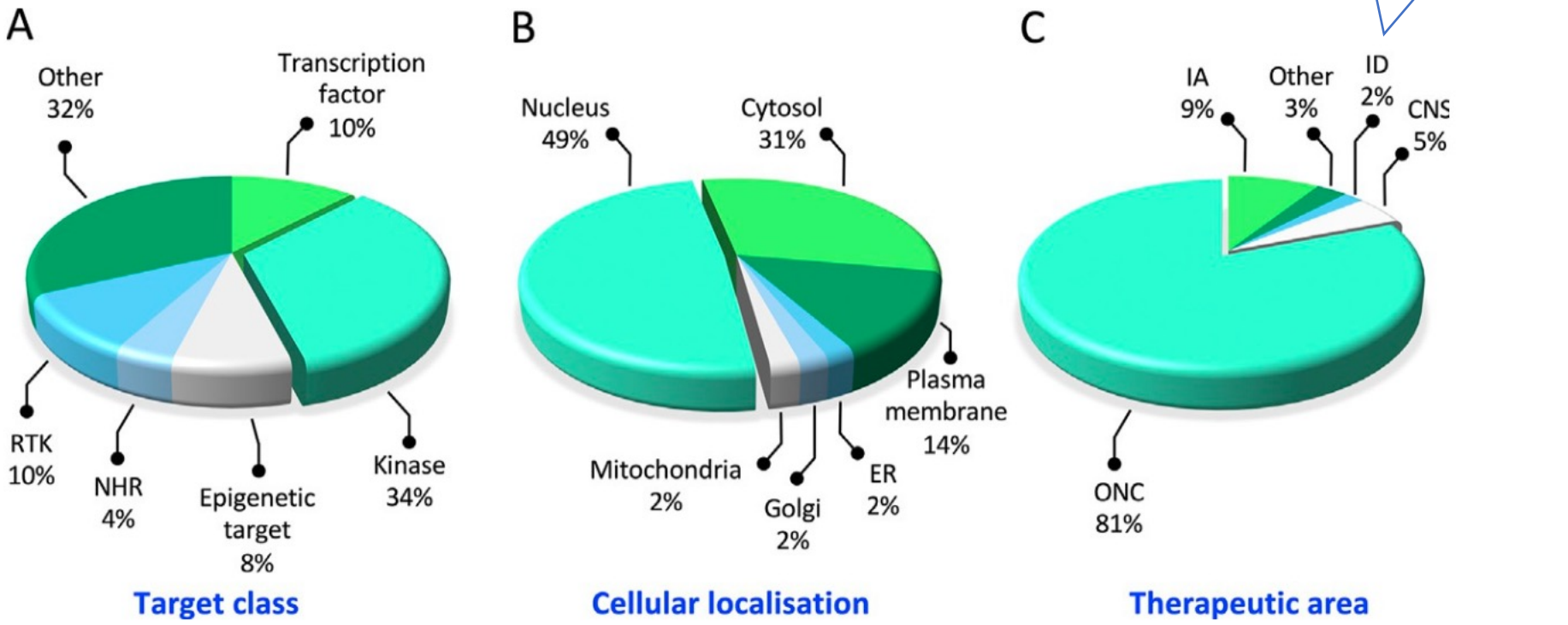
Table 1 | **Selected degraders in and approaching the clinic**

Drug	Sponsor	Properties	Lead indication	Status
Heterobifunctional degraders (PROTACs, BiDACs, etc.)				
ARV-110	Arvinas	Androgen receptor degrader	Prostate cancer	Phase II
ARV-471	Arvinas	Oestrogen receptor degrader	Breast cancer	Phase II
ARV-766	Arvinas	Androgen receptor degrader	Prostate cancer	Phase I in 2021
AR-LDD	Bristol Myers Squibb	Androgen receptor degrader	Prostate cancer	Phase I
DT2216	Dialectic	BCL-XL degrader	Liquid and solid cancers	Phase I
KT-474	Kymera/Sanofi	IRAK4 degrader	Autoimmune including AD, HS and RA	Phase I
KT-413	Kymera	IRAK4 degrader with IMiD activity	MYD88-mutant DLBCL	Phase I in 2H2021
KT-333	Kymera	STAT3 degrader	Liquid and solid tumours	Phase I in 2H2021
NX-2127	Nurix	BTK degrader with IMiD activity	B cell malignancies	Phase I
NX-5948	Nurix	BTK degrader	B cell malignancies and autoimmune	Phase I in 2H2021
CG001419	Cullgen	TRK degrader	Cancer and other diseases	IND in 2021
CFT8634	C4 Therapeutics	BRD9 degrader	Synovial sarcoma	IND in 2H2021
FHD-609	Foghorn	BRD9 degrader	Synovial sarcoma	IND in 1H2021

Asher Mullard, Nature reviews | Drug Discovery ,2021

Application of TPD

Distribution of proteins degraded by PROTACs



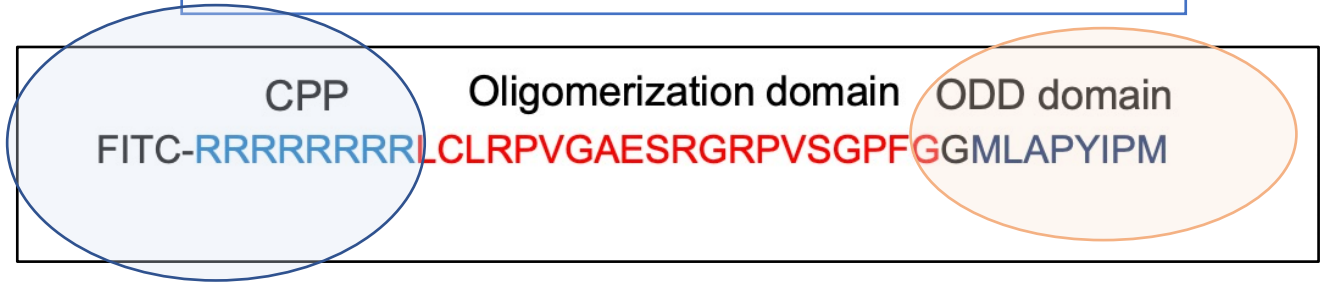
M. Maneiro et al, Progress in Medicinal Chemistry, 2021

TPD as Antiviral Therapeutics

- Peptide-based PROTAC
- Poly-arginine cell-penetrating peptide

Hepatitis B virus (HBV)

- Chronic infection with HBV
- Major risk for hepatocellular carcinoma (HCC)



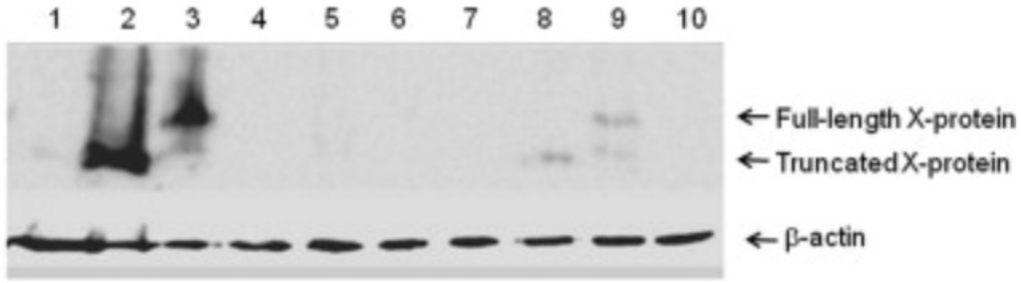
ligand for X-Protein

ligand for VHL

X-Protein

HBx

- Essential for viral replication
- HCC



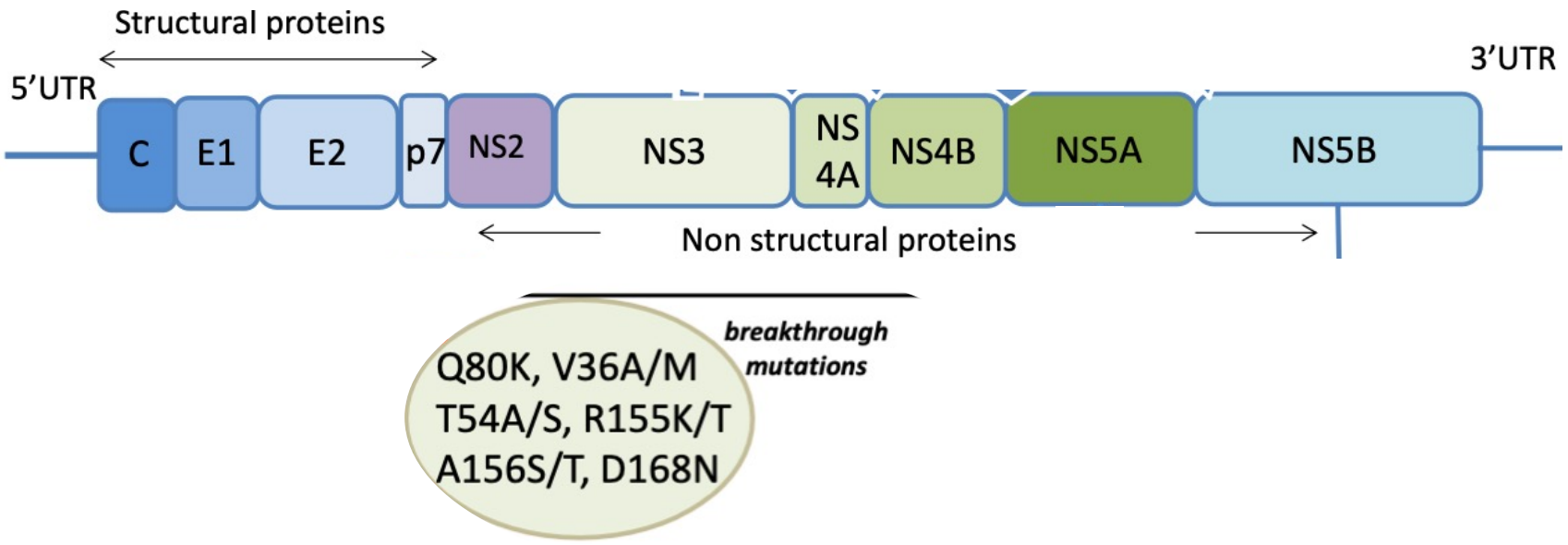
lane 1:untransfected
lanes 2, 5, and 8 :transfected with plasmids encoding **truncated forms** of the X-protein
lanes 3, 6, and 9 : transfected with plasmids encoding **full-length forms** of the X-protein
lanes 4, 7, and 10 : transfected with the control plasmid
lanes 1–4 :left untreated
treated with the PROTAC containing the HIF-1α ODD domain located N-terminally (lanes 5–7) or **C-terminally (lanes 8–10)**.

Provided evidence that peptide-based PROTAC destroyed the X-protein in HepG2 cells effectively

Montrose， et al Biochemical and Biophysical Research Communications， 2014

TPD as Antiviral Therapeutics

Hepatitis C virus (HCV) genome



NS3/4A inhibitors
Telaprevir, Boceprevir,
Simeprevir, Faldaprevir, Asunaprevir

Encounter drug-resistant issues

Asma Ahmed, Viruses 2015

TPD as Antiviral Therapeutics

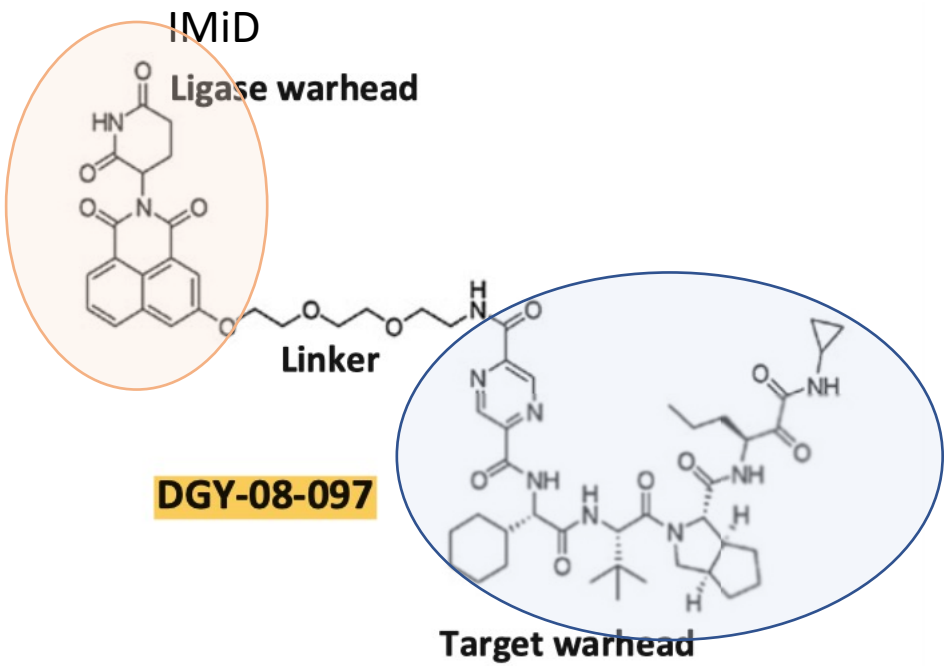
nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-11429-w OPEN

Small molecule degraders of the hepatitis C virus protease reduce susceptibility to resistance mutations

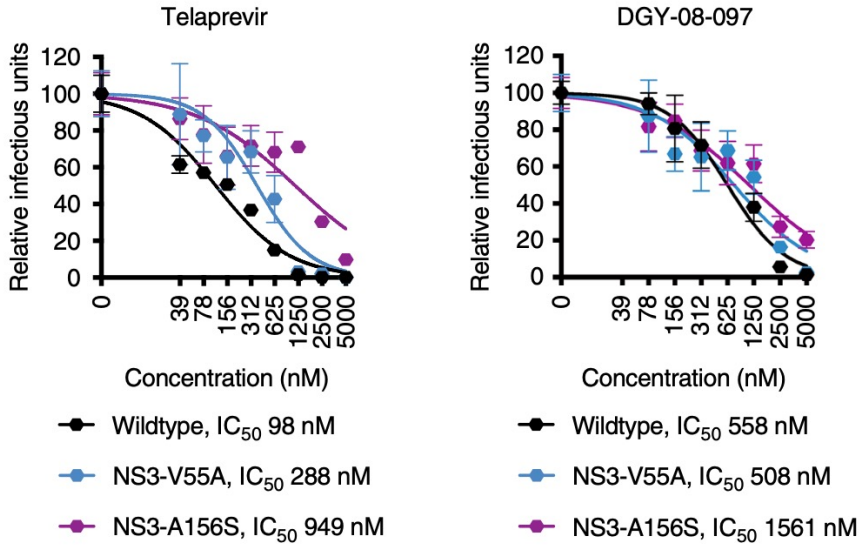
Mélissanne de Wispelaere[1,4], Guangyan Du[2,3,4], Katherine A. Donovan[2,3], Tinghu Zhang[2,3], Nicholas A. Eleuteri[3], Jingting C. Yuan[3], Joann Kalabathula[3], Radosław P. Nowak[2,3], Eric S. Fischer[2,3], Nathanael S. Gray[2,3] & Priscilla L. Yang[1]



Telaprevir

Telaprevir

- A reversible-covalent inhibitor that binds to the HCV NS3/4A protease active site

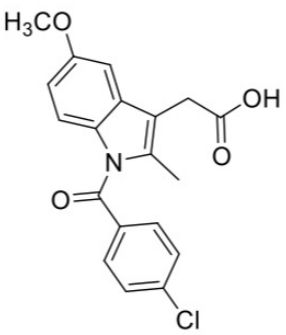


- **Inhibit telaprevir-resistant HCV**

TPD as Antiviral Therapeutics

Anti-CoVs

a)



indomethacin

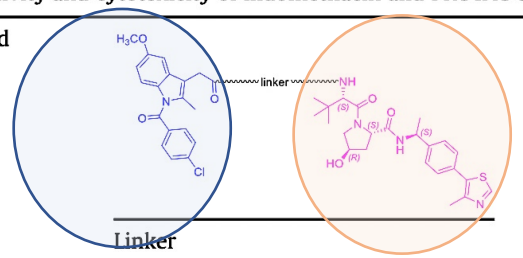
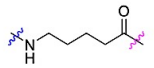
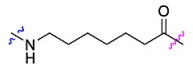
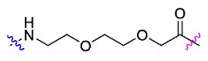
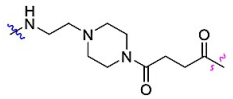


Table 1
Antiviral activity and cytotoxicity of indomethacin and PROTAC compounds against SARS-CoV-2.

Compound	Linker	CC_{50}[a] (μM)	SARS-CoV-2/NL/2020 EC_{50}[b] (μM) (CI)[c]	SI[d]	SARS-CoV-2/Padova/2021 EC_{50} (μM) (CI)	SI
INM	–	>500	94.9 (53.3–175.2)	>5	N.D.	–
2		>200	>50	>4	>50	>4
3		>200	18.1 (7.1–46.4)	>11	25.4 (9.8–69.2)	>8
4		>200	>50	>4	>50	>4
5		>250	21.5 (11.2–43.4)	>12	29.8 (14.5–41.9)	>8
RMV	–	373 ± 20	0.15 (0.11–0.20)	2487	0.25 (0.18–0.36)	1492

- INM-based PROTACs 3 and 5 demonstrated an almost 5-fold improved ability in inhibiting viral replication

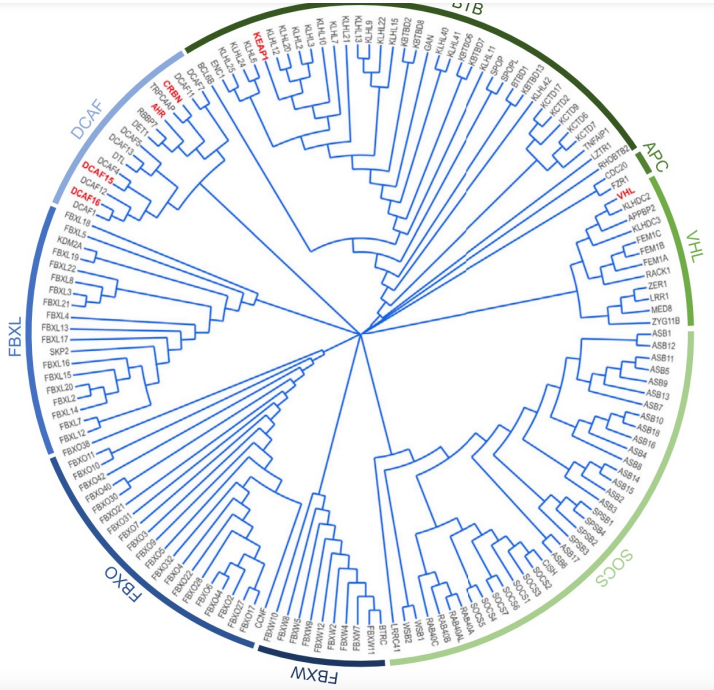
- Molecular modelling studies support human PGES-2 as a potential target of INM- based antiviral PROTACs

Jenny Desantis et al, European Journal of Medicinal Chemistry 2021

Challenges of TPD

1.new E3 ligase for PROTAC development

- few harnessed for TPD



Receptor protein
eg. Cereblon (CRBN)
Von Hippel–Lindau (VHL)
Cell inhibitor of apoptosis protein(cIAP)
Mouse double minute 2 homolog (MDM2)

2. How to rationally design PROTACs are still unclear
eg.hook effect， MW

Kannt et al, Cell Chemical Biology (2021)

Thanks for listening！

Acknowledgments！