A PK/PD Model for the Assessment and Optimization of PROTACs

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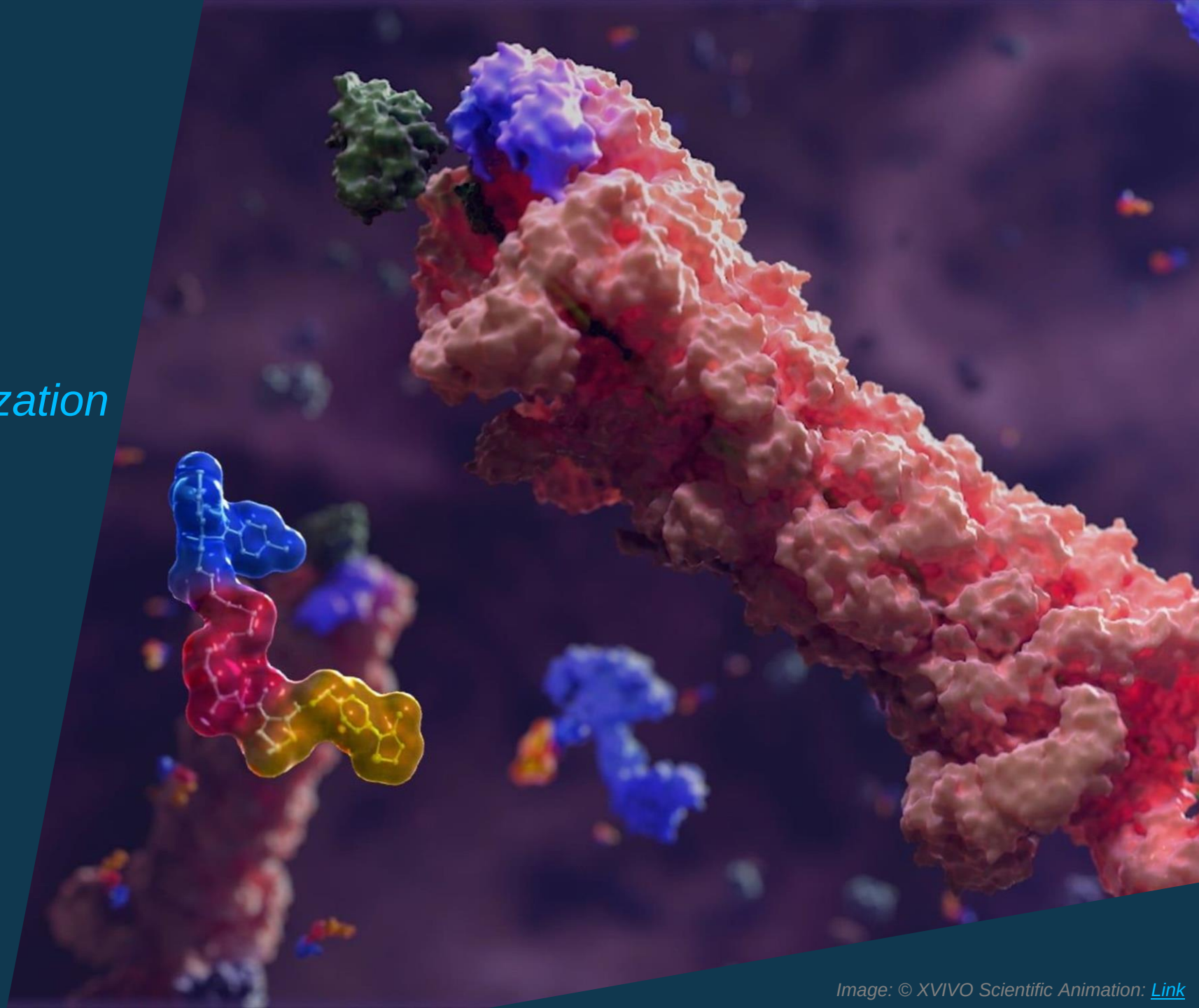
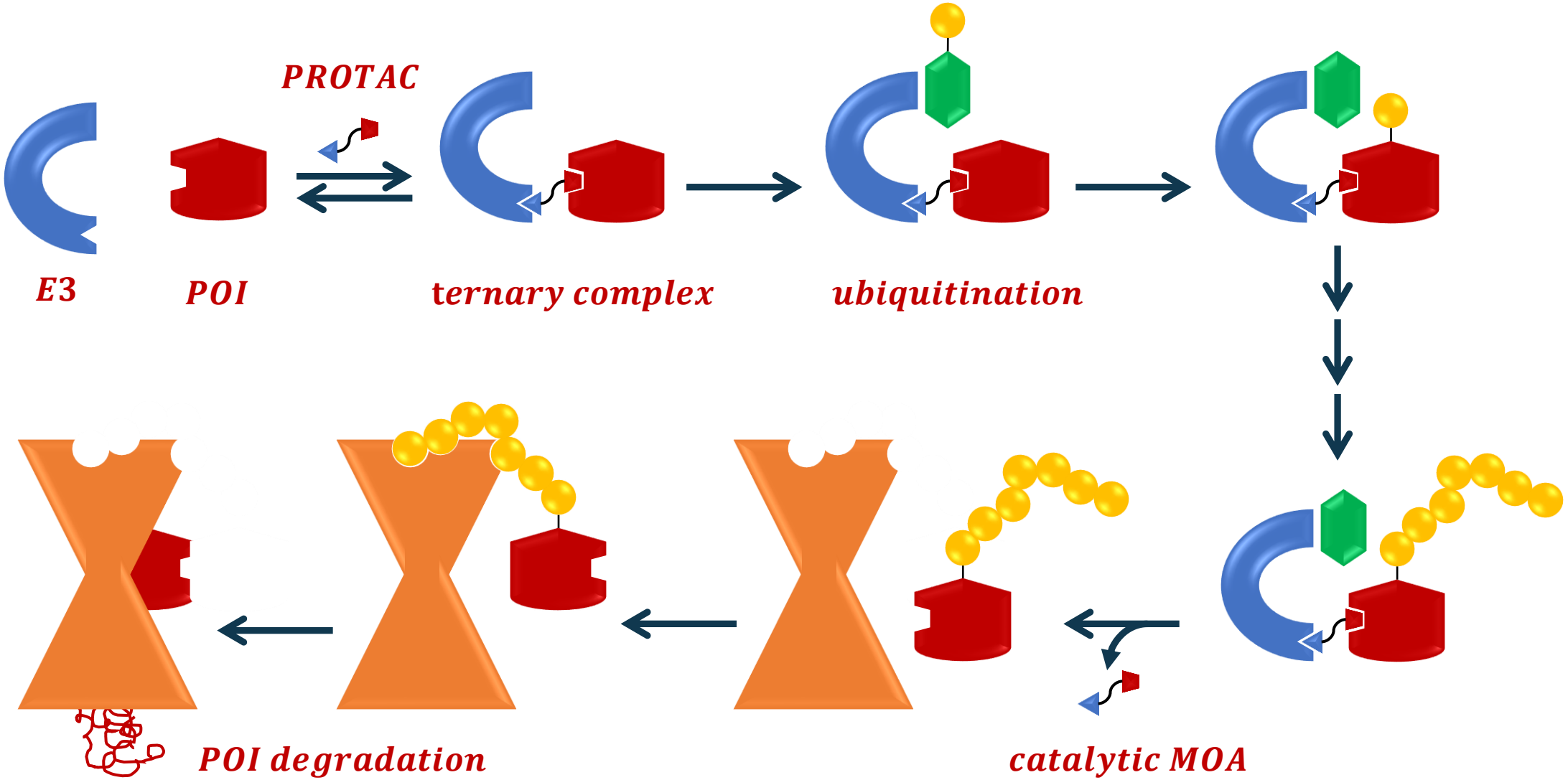


Image: © XVIVO Scientific Animation: Link

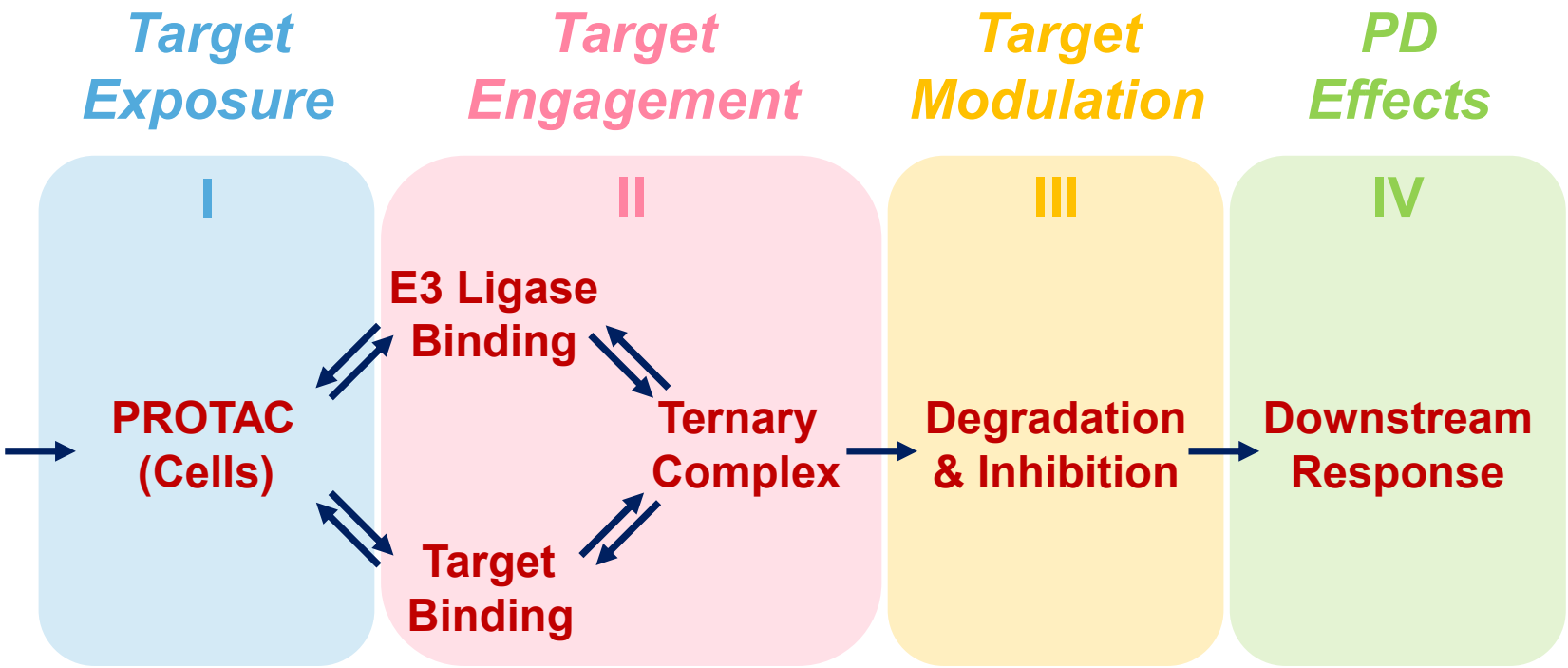
Introduction

PROTAC – Mechanism of Action



Approach

Applying the four pillars concept to Proteolysis Targeting Chimeras [1]



➢ Basis for a **mechanistic modeling** framework that addresses three key questions

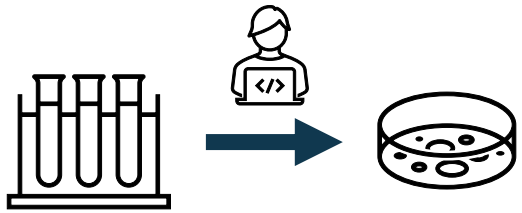
[1] *Nowak & Jones (2021) SLAS Discov.* DOI: 10.1177/2472555220979584

Overview

Preclinical PK/PD modeling plays a crucial role in three distinct translational steps

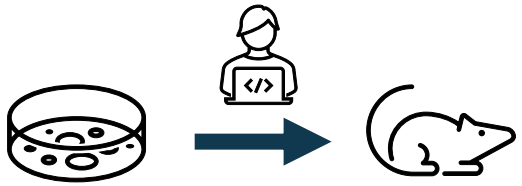
1) Translation from biochemical to cellular level

   - How to increase degradation potency?



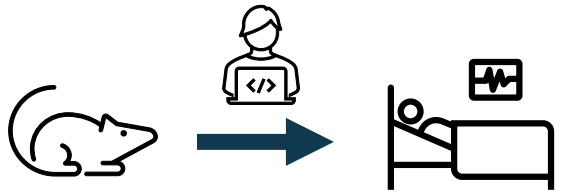
2) Translation from cellular level to animal model

   - Which compounds to take *in vivo*?



3) Translation from animal model to human patients

   - What is the relevant dose in humans?



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Preclinical PK/PD modeling plays a crucial role in three distinct translational steps [2]

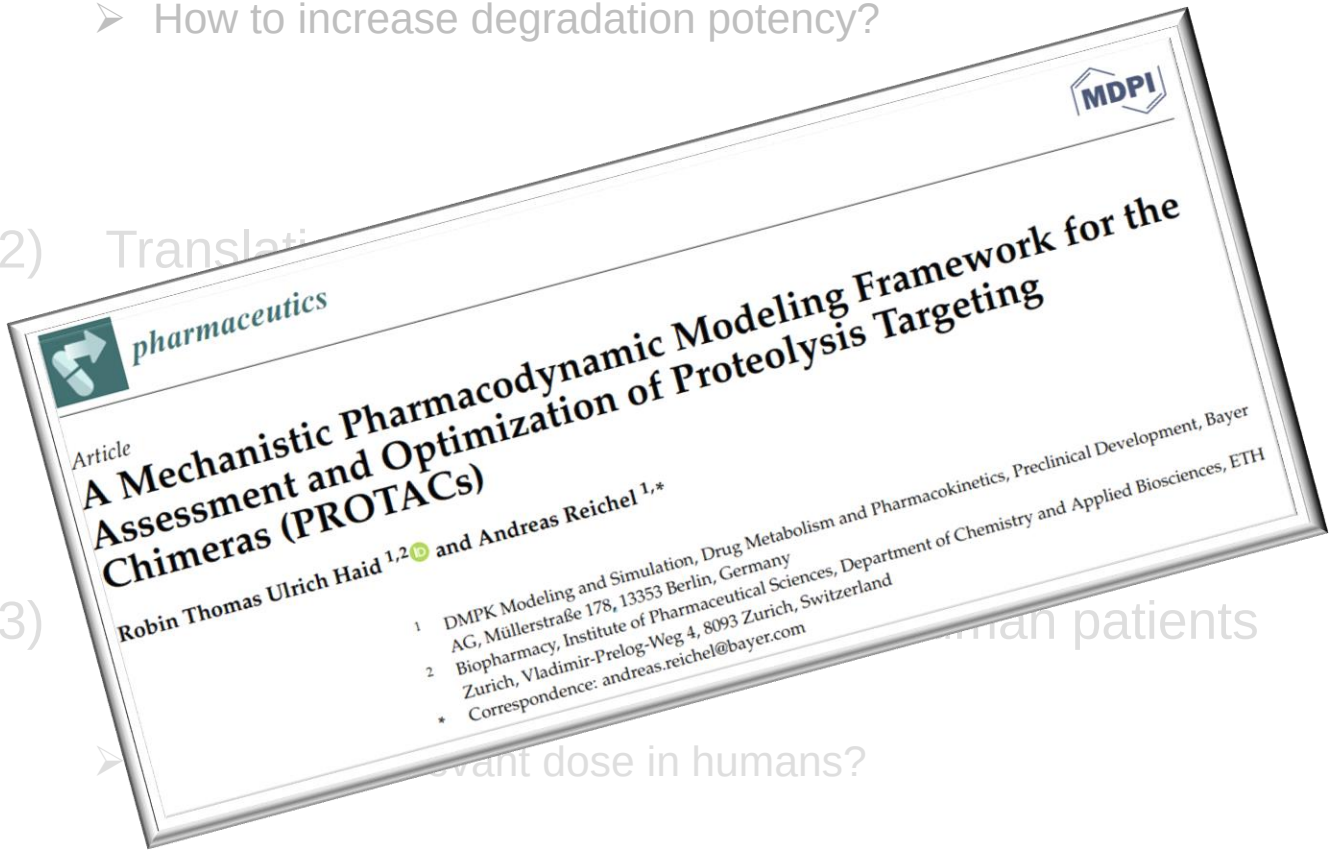
1) Translation from biochemical to cellular level

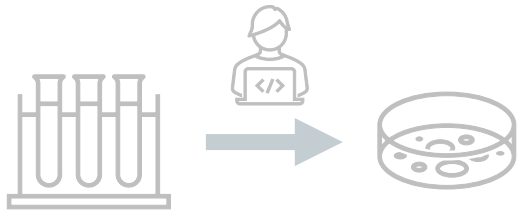
➢ How to increase degradation potency?

2) Translati

3) human patients

➢ evant dose in humans?





SCAN ME

[2] paper: *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195

Overview

Already with in vitro data, different tasks require different models

I) Assessing PROTACs as Degraders

- How much degradation is there?

II) Model-Informed Optimization of PROTACs

- How to increase degradation?

III) Deriving a Target Value for Degradation

- How much degradation is necessary?

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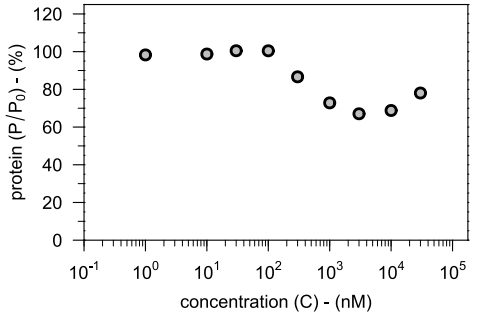

hook model

- $D_{\max}$
- DC_{50}
- $DC_{\max}$

II) Model-Informed Optimization of PROTACs

- How to increase degradation?

III) Deriving a Target Value for Degradation

- How much degradation is necessary?

I) *Assessing PROTACs as Degraders*

Describe the concentration-response profile mathematically [3]

	$E_{\max}$ model
$D_{\max}$ (%)	41
DC_{50} (nM)	665
$DC_{\max}$ (nM)	NA

$D_{\max}$ … max. extent of degradation
DC_{50} … conc. of half-max. deg.
$DC_{\max}$ … conc. of max. deg

→ *cf.* $E_{\max}$, EC_{50} etc. with relevant **e**ffect being **d**egradation

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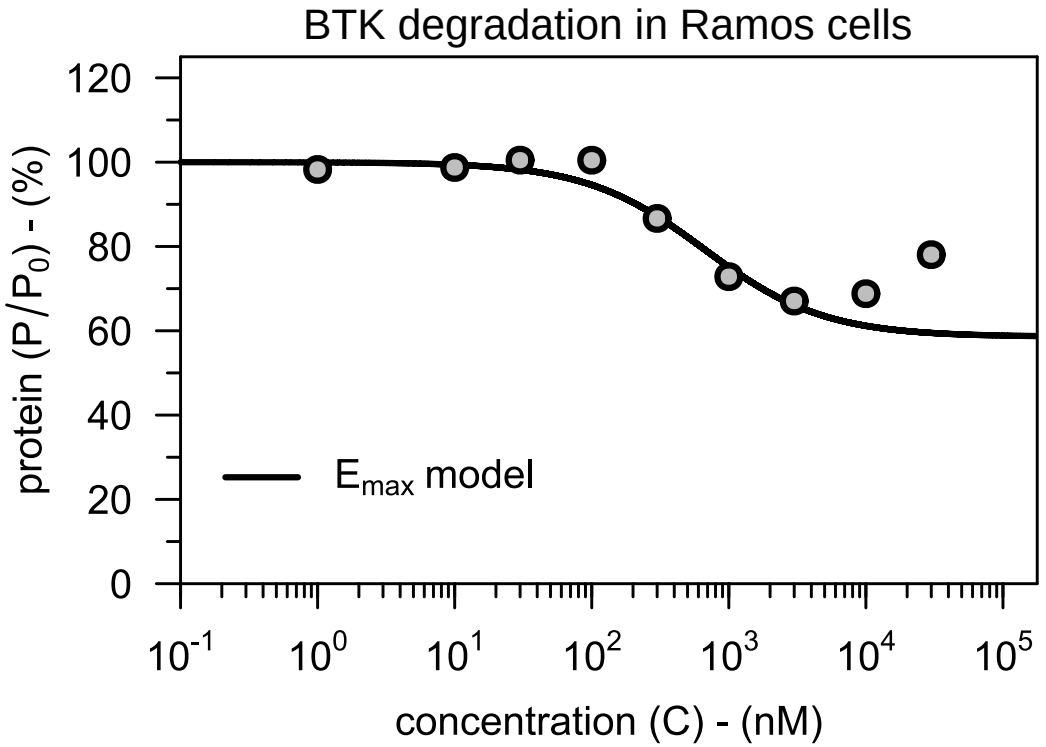
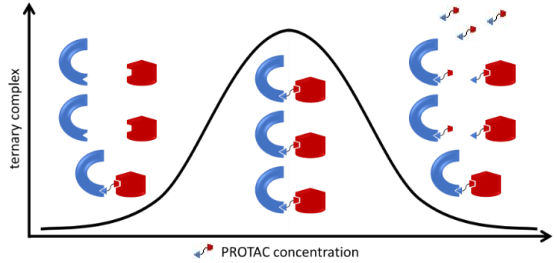


→ *hook effect:* @ high conc., the non-degrading binary complexes dominate

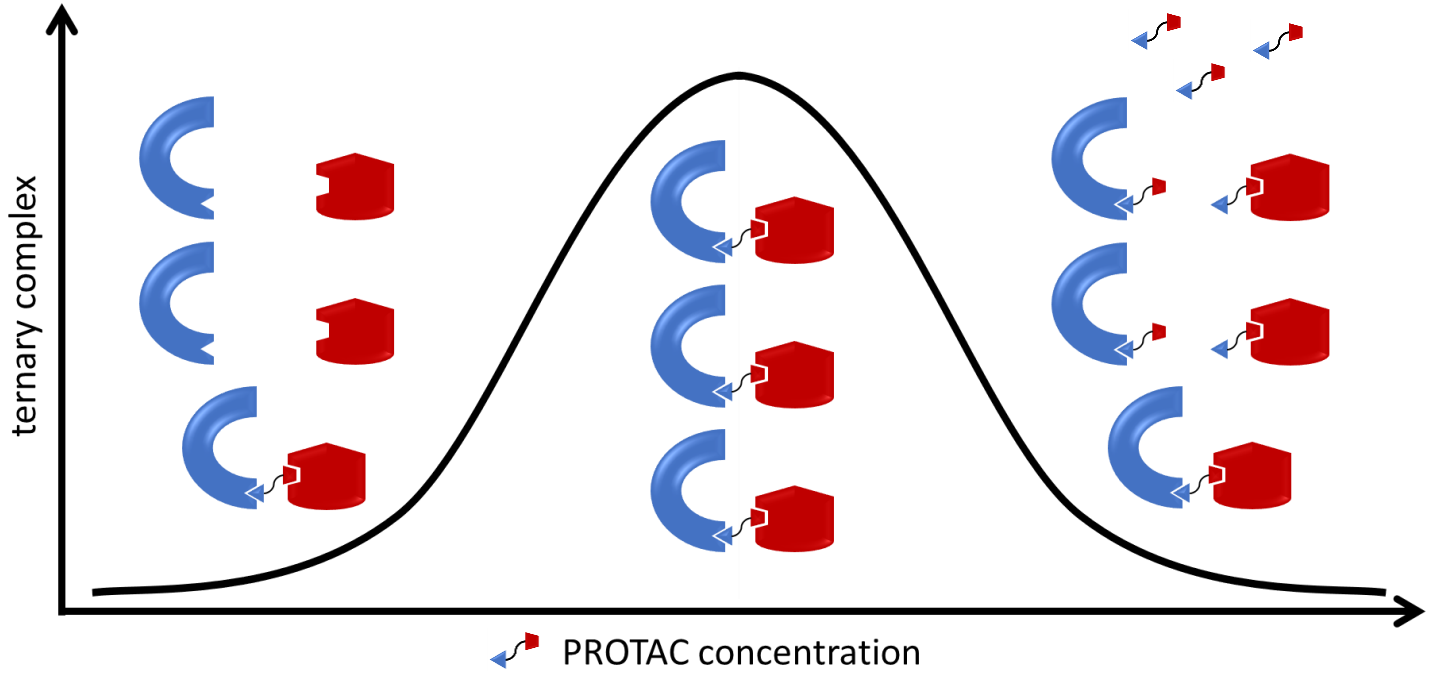


➢ The $E_{\max}$ model cannot account for the **hook effect** at high drug concentrations

[3] data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115

I) *Assessing PROTACs as Degraders*

Describe the concentration-response profile mathematically [3]



- *Hook effect*: at **high concentrations** PROTACs mainly form **binary** instead of ternary complexes

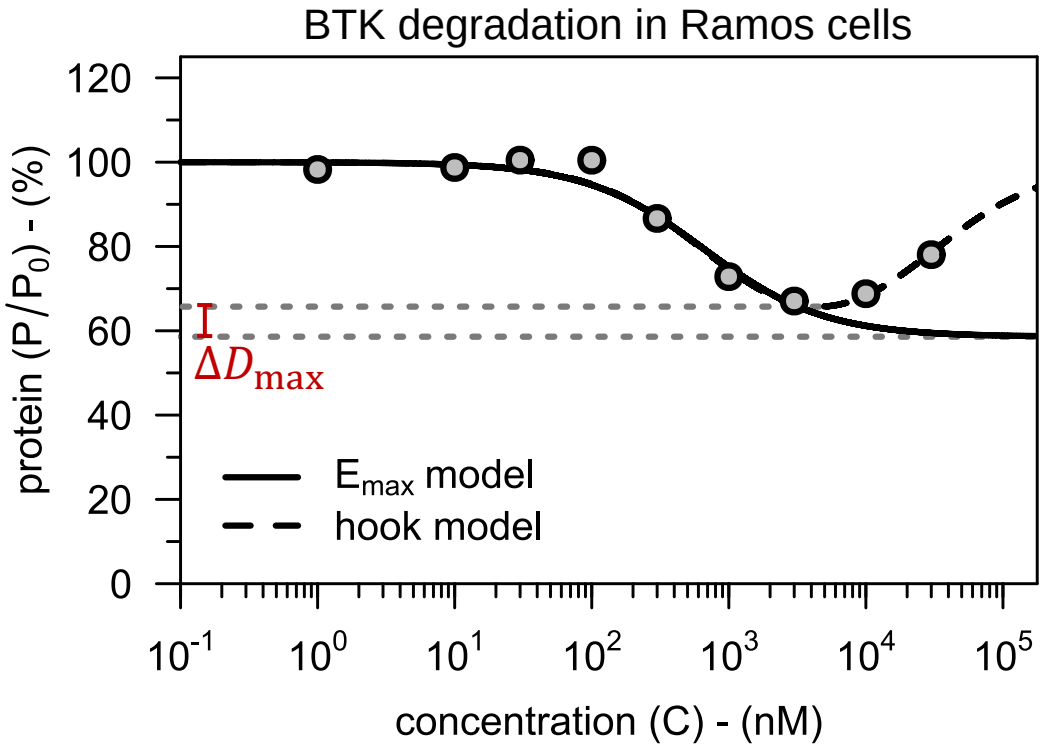
[3] data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115

I) *Assessing PROTACs as Degraders*

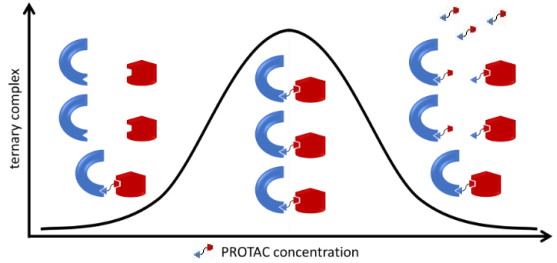
Describe the concentration-response profile mathematically [3]

	E_{max} model	*hook* model
D_{max} (%)	41	34
DC_{50} (nM)	665	464
DC_{max} (nM)	NA	4,550

→ same data were analyzed using different models



→ *hook effect:* @ high conc., the non-degrading binary complexes dominate



➢ The *hook model* [2] fits **all the data** and gives an **accurate** estimate of D_{max}

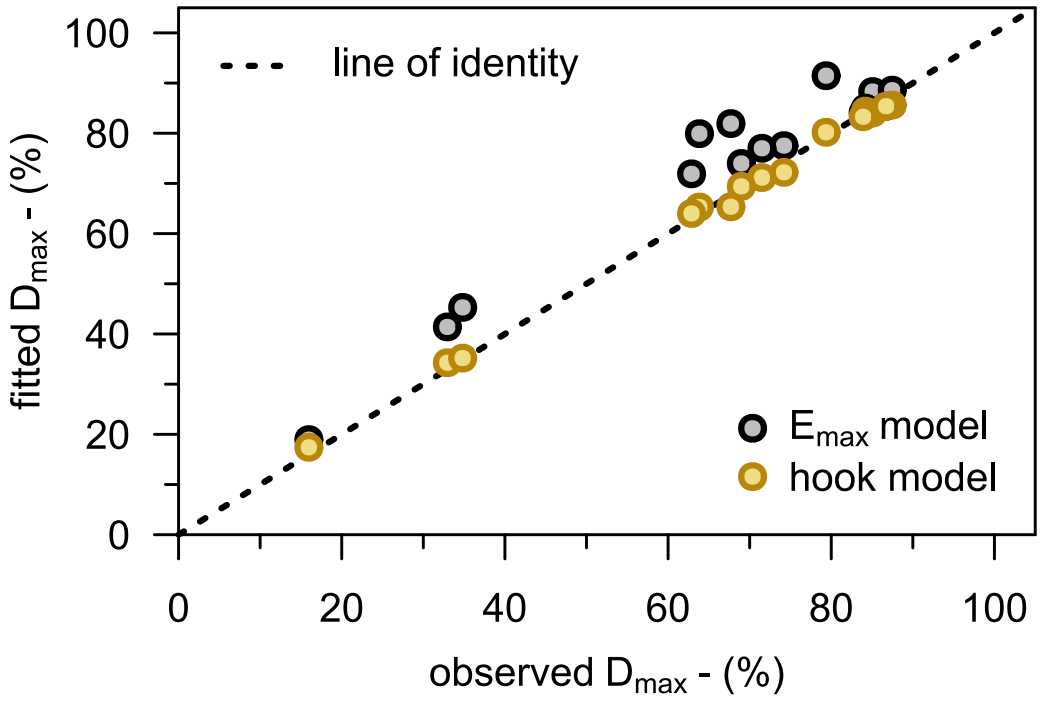
[2] *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195
[3] data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115

I) *Assessing PROTACs as Degraders*

Describe the concentration-response profile mathematically [3]

E_{max}	obs.	*hook*
H2	F1	F1
F1	H1	H1
D1	D1	E1
H1	G1	D1
E1	E1	G1
G1	I1	I1
I1	H2	H2
D2	F2	F2
C1	G2	G2
F2	I2	I2

→ the hook model is better at ranking PROTACs



→ a total of 16 conc.-deg. profiles were analyzed with both models each

➢ The E_{max} model tends to **overestimate** D_{max} and results in **flawed** compound **rankings**

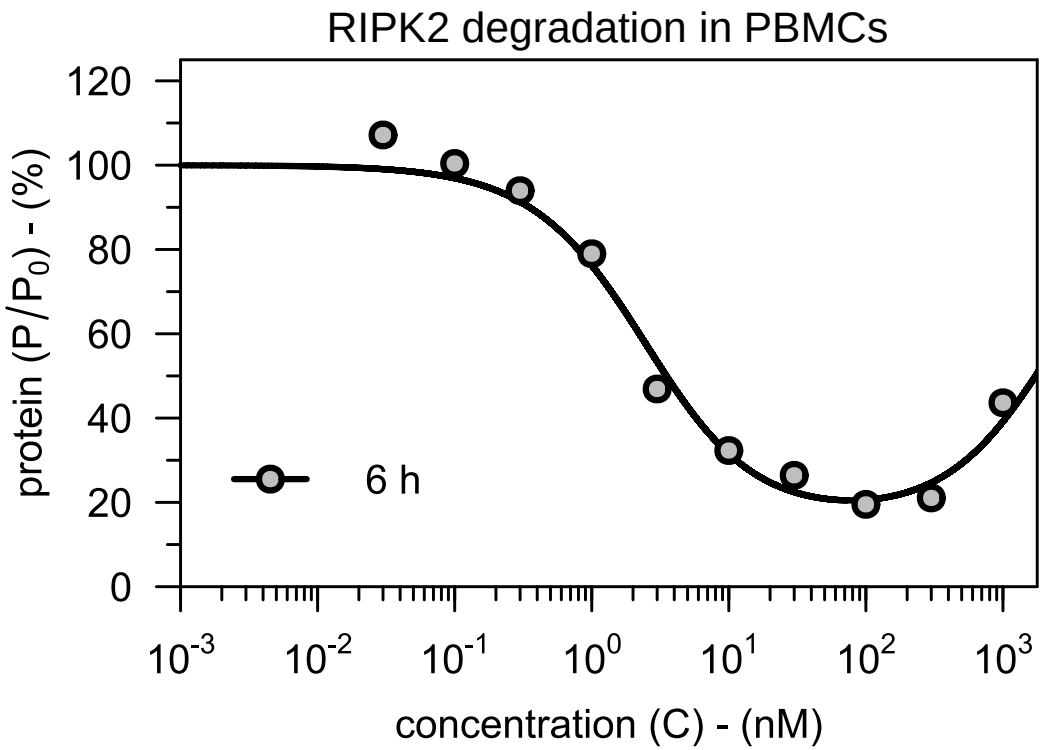
[3] data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115

I) *Assessing PROTACs as Degraders*

Account for the impact of the experimental incubation time [4,5]

	6 h
D_{max} (%)	79.8
DC_{50} (nM)	2.19
DC_{max} (nM)	79.8

→ apparent values (uncertainty not shown)



1) the *extended hook model* [2] is fitted to degradation data observed after 6 h

➢ The decision, for **how long** cells are incubated *in vitro* is somewhat **arbitrary**

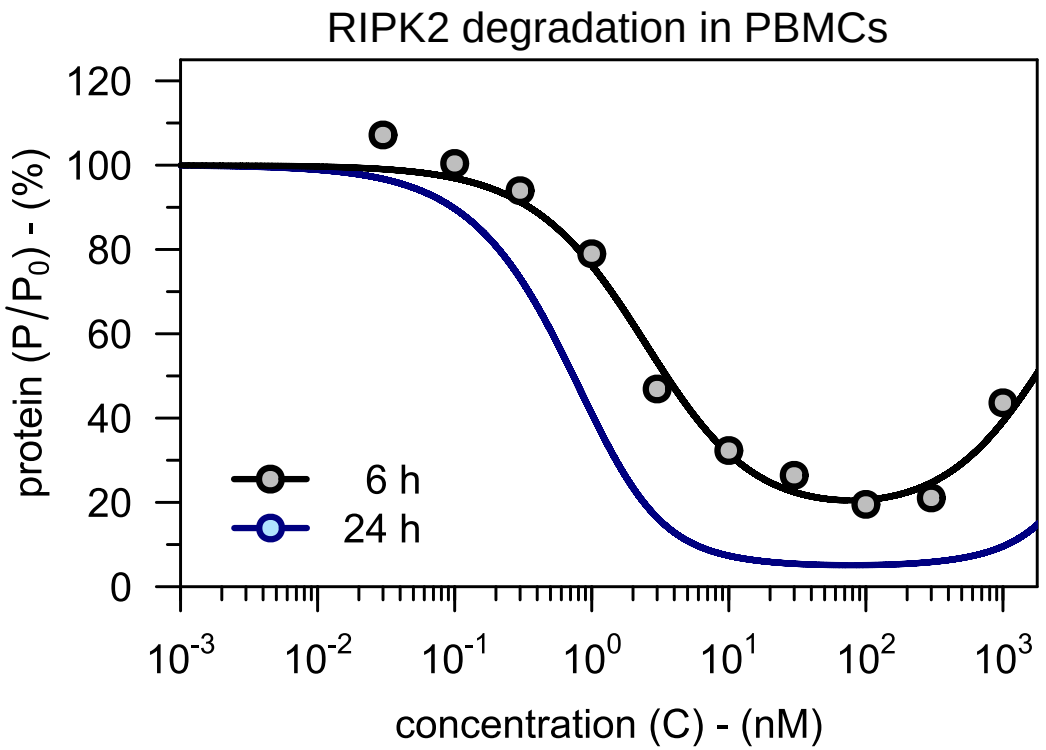
2. *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195
4. data: *Mares et al. (2020) Commun. Biol.* DOI: 10.1038/s42003-020-0868-6
5. data: *Mathieson et al. (2018) Nat. Commun.* DOI: 10.1038/s41467-018-03106-1

I) *Assessing PROTACs as Degraders*

Account for the impact of the experimental incubation time [4,5]

	6 h	24 h
D_{max} (%)	79.8	95.5
DC_{50} (nM)	2.19	0.65
DC_{max} (nM)	79.8	73.7

→ extent of degradation increases over time



1) the *extended hook model* [2] is fitted to degradation data observed after 6 h

2) using the protein's baseline half-life ($t_{½,P} = 45$ h), deg. after 24 h is predicted

➢ The choice of **incubation time** influences the extent of **degradation** that is **observed**

2. *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195
4. data: *Mares et al. (2020) Commun. Biol.* DOI: 10.1038/s42003-020-0868-6
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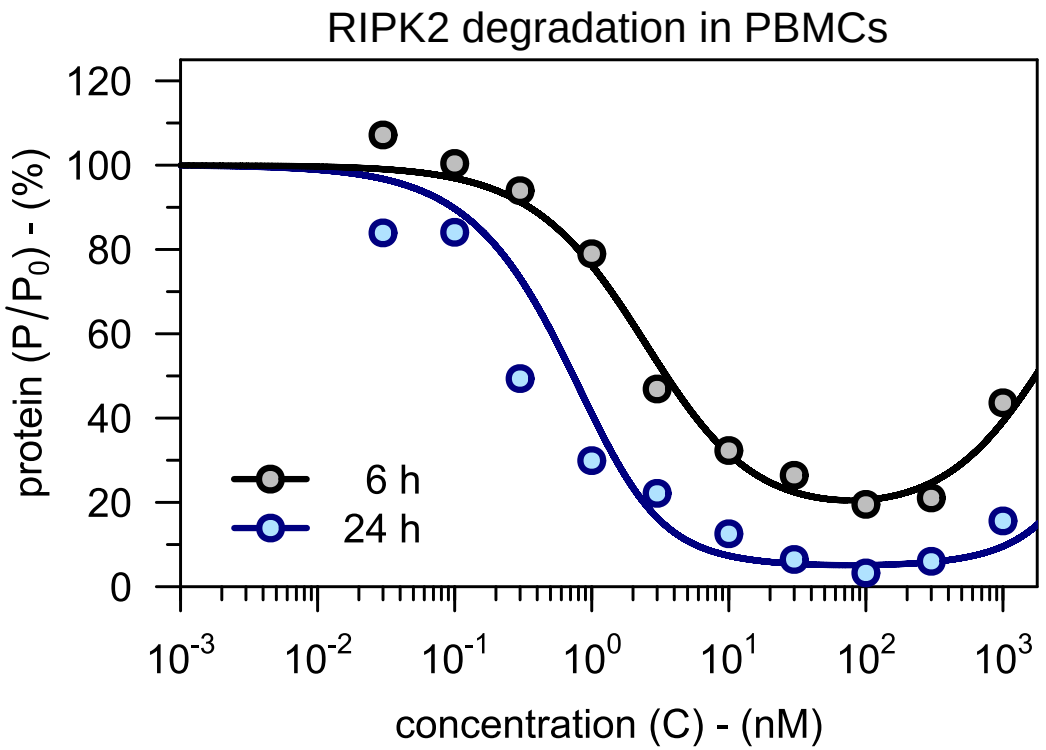
I) *Assessing PROTACs as Degraders*

Account for the impact of the experimental incubation time [4,5]

	24 h	steady state
D_{max} (%)	95.5	94.9
DC_{50} (nM)	0.65	0.29
DC_{max} (nM)	73.7	68.9

→ incubation time is most critical for potency (DC_{50})



1) the *extended hook model* [2] is fitted to degradation data observed after 6 h
2) using the protein's baseline half-life ($t_{½,P} = 45$ h), deg. after 24 h is predicted
3) the predicted profile (24 h) is confirmed experimentally to validate the approach

➢ The *extended hook model* [2] estimates the true (i.e. **steady-state**) degradation parameters

[4] data: *Mares et al. (2020) Commun. Biol.* DOI: 10.1038/s42003-020-0868-6
[5] data: *Mathieson et al. (2018) Nat. Commun.* DOI: 10.1038/s41467-018-03106-1

I) *Assessing PROTACs as Degraders*

Account for the impact of the experimental incubation time

→ the necessary incubation time depends on:
  i) protein half-life ($t_{½,P}$)
  ii) drug effectiveness (D_{max})

→ as a rule of thumb, choose incubation times to roughly match POI half-life

→ too short an incubation makes the cpd. seem worse than it is

	D_{max} (%)				
$t_{½,P}$ (h)	70	80	90	95	99
4	5	4	4	3	3
12	13	12	10	9	9
24	26	23	20	18	17
48	52	45	39	36	33
96	103	90	77	71	66

→ incubation for 24 h is long enough for the green cells, but NOT for the yellow ones

➢ Incubation for **24 h** might **NOT** be **sufficient** to observe the steady-state parameters

Overview

Already at the in vitro stage, different tasks require different models

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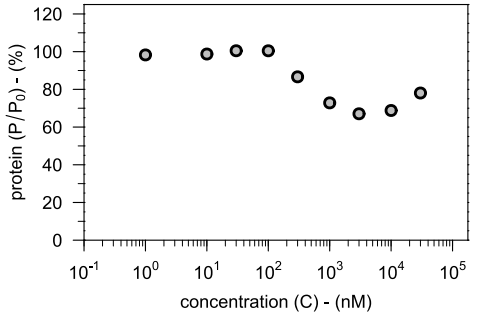
- How much degradation is there?

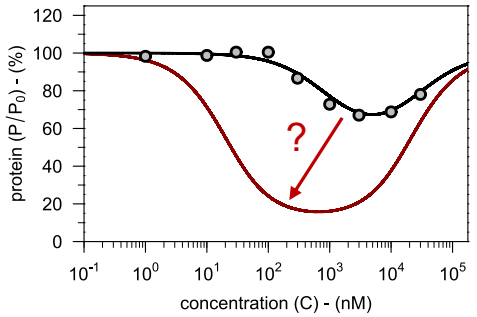


hook model

- $D_{\max}$
- DC_{50}
- $DC_{\max}$

II) Model-Informed Optimization of PROTACs

- How to increase degradation?



k_{cat} model

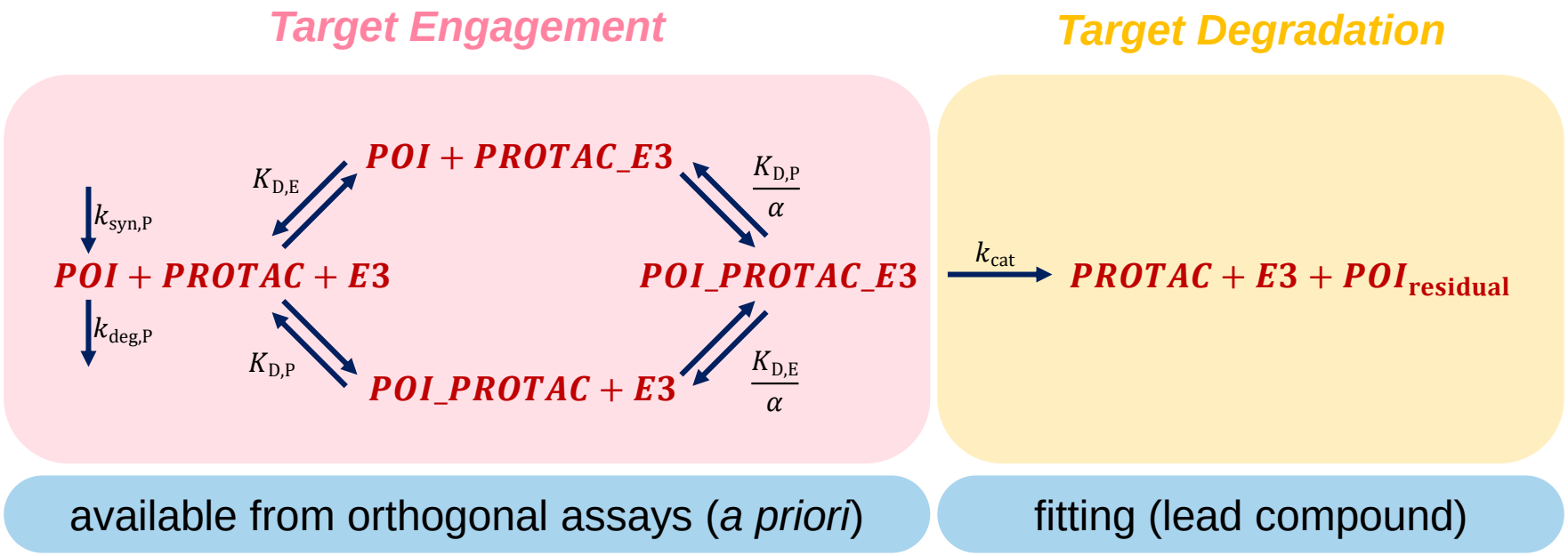
- $K_{\mathrm{D,P}}$ ↓
- $K_{\mathrm{D,E}}$ ↓
- α ↑

III) Deriving a Target Value for Degradation

- How much degradation is necessary?

II) *Model-Informed Optimization of PROTACs*

Determine the biochemical parameters governing target degradation



➢ The k_{cat} *model* [2] integrates **compound-specific** parameters with **physiological** parameters

[2] *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195

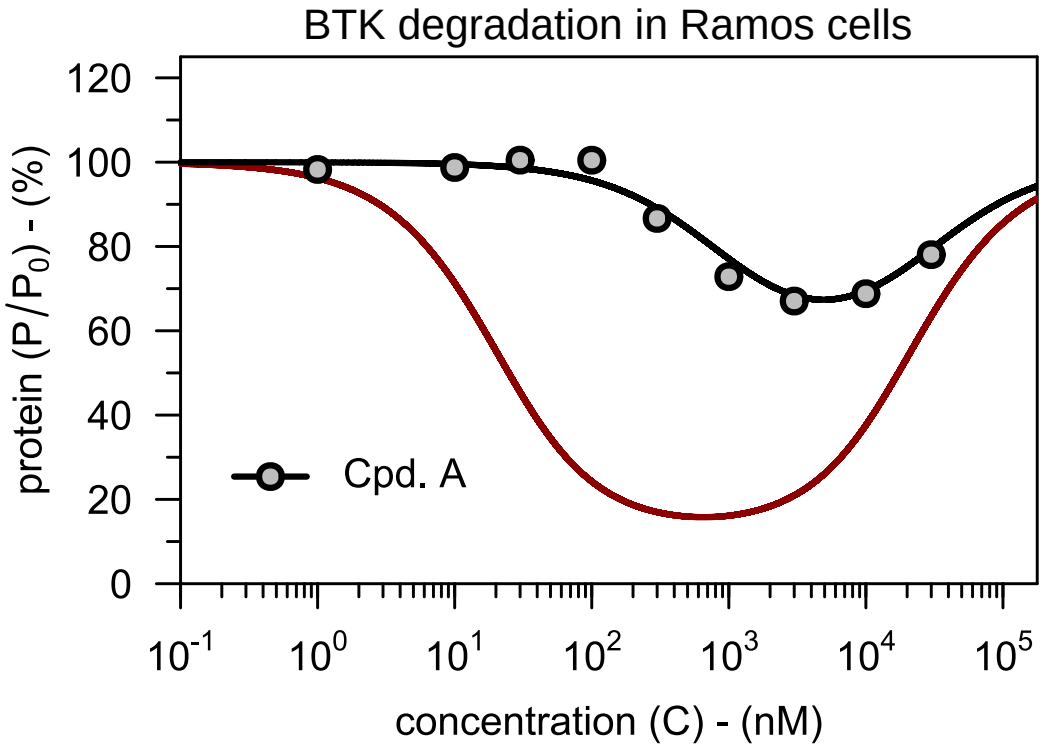
II) *Model-Informed Optimization of PROTACs*

Predict target degradation from a compound's binding affinities [3,6]

	Cpd. A
$K_{D,P}$ (nM)	1,535
$K_{D,E}$ (nM)	15,700
α (1)	0.89

$K_{D,P}$ … affinity for target protein (POI)
$K_{D,E}$ … affinity for E3 ligase (enzyme)
α … interaction of POI and E3 ligase

→ three binding partners, hence three equilibrium constants



1) the binding affinities [7] are used to fit the observed degradation data

➢ The first step in **improving** a bad PROTAC is **identifying** its shortcomings

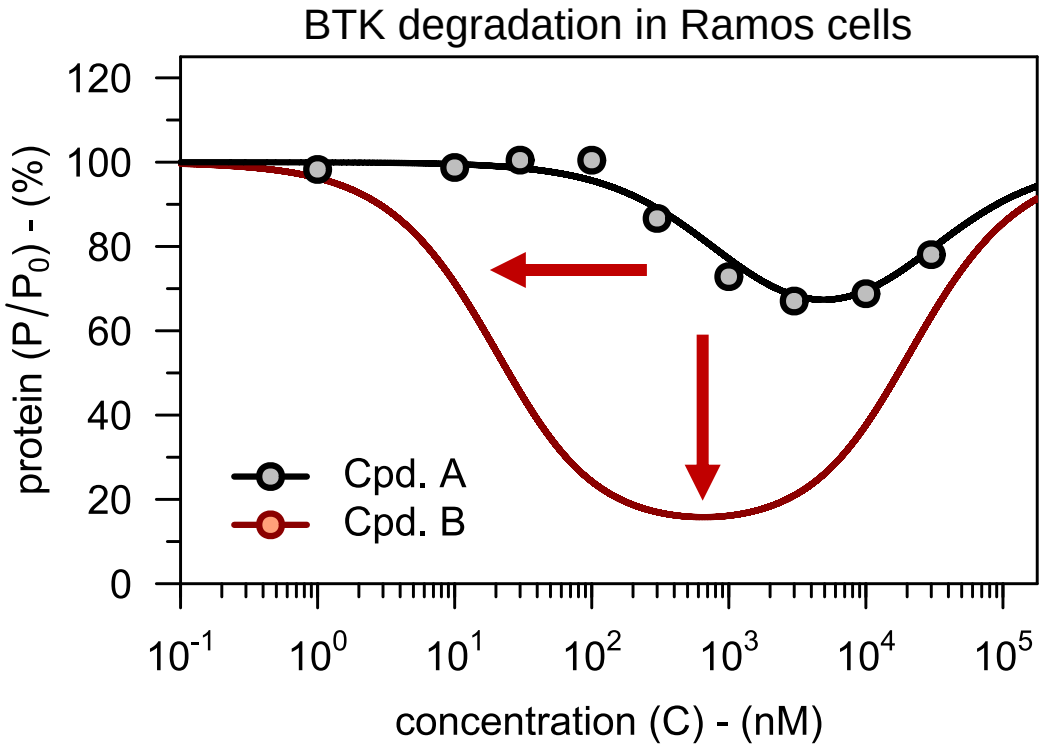
[3.] data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115
[6.] data: *Bradshaw et al. (2015) Nat. Chem. Biol.* DOI: 10.1038/nchembio.1817
[7.] related poster: *Kim et al. (2023) PAGE Conference* Link: https://tinyurl.com/4z45wv8r

II) *Model-Informed Optimization of PROTACs*

Predict target degradation from a compound's binding affinities [3,6]

	Cpd. A	Cpd. B
$K_{D,P}$ (nM)	1,535 →	×10
$K_{D,E}$ (nM)	15,700 →	×5
α (1)	0.89 →	×1.5

→ higher affinities needed for desired degradation



1) the binding affinities [7] are used to fit the observed degradation data

2) the resulting model tells us, how binding affinities have to be improved

➢ Due to its **multiparametric** nature (three affinities), PROTAC optimization is often **non-intuitive**

[3] data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115
[6] data: *Bradshaw et al. (2015) Nat. Chem. Biol.* DOI: 10.1038/nchembio.1817
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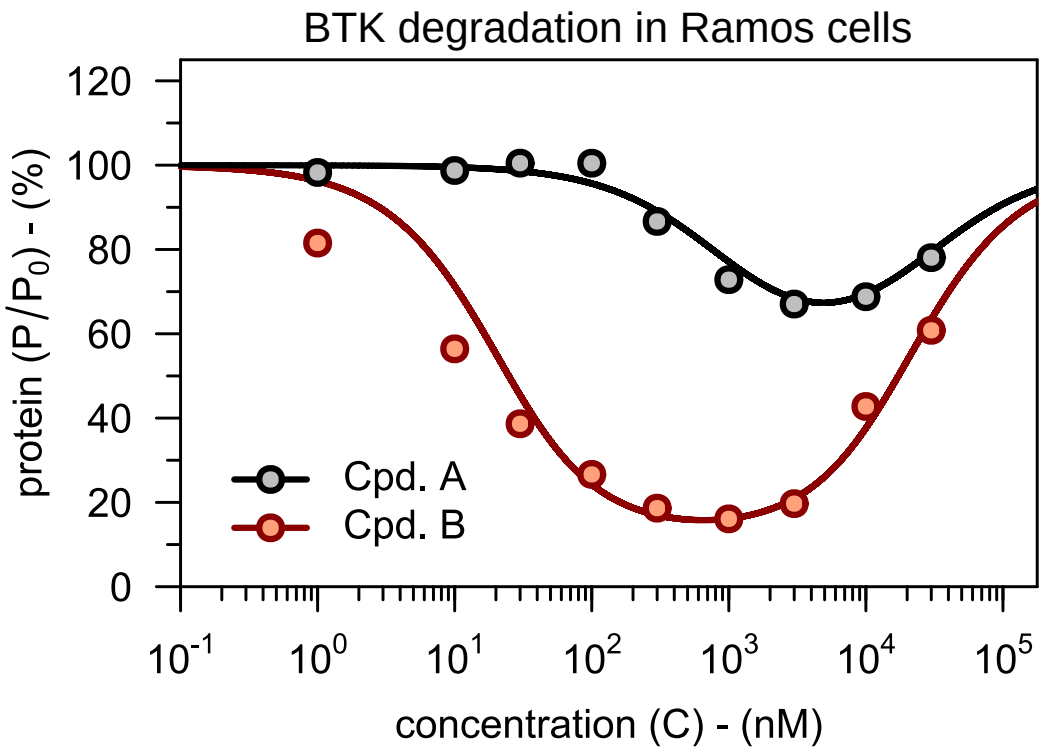
II) *Model-Informed Optimization of PROTACs*

Predict target degradation from a compound's binding affinities [3,6]

	Cpd. A	Cpd. B
$K_{D,P}$ (nM)	1,535	138
$K_{D,E}$ (nM)	15,700	3,100
α (1)	0.89	1.34

→ all three binding affinities need to be considered



1) the binding affinities [7] are used to fit the observed degradation data

2) the resulting model tells us, how binding affinities have to be improved

3) the prediction (Cpd. B) is validated with experimental data from a real PROTAC

➢ The k_{cat} *model* [2] **guides** medicinal **chemistry** during compound **optimization**

2. *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195
3. data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115
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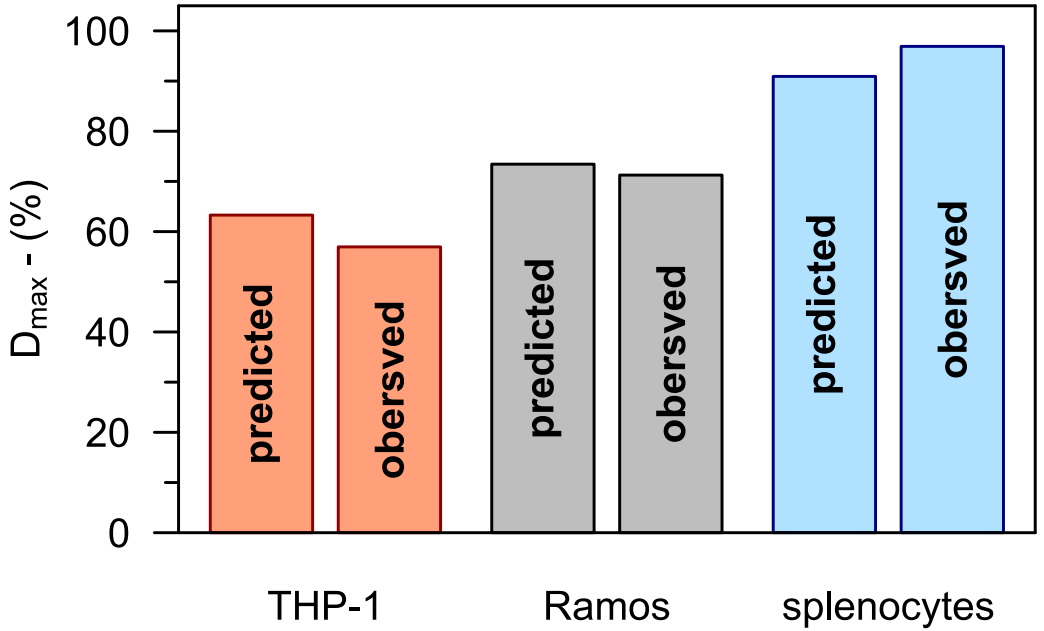
II) *Model-Informed Optimization of PROTACs*

Evaluate the impact of physiological parameters on target degradation [3,5,6]

	E_0 (nM)	$t_{½,P}$ (h)
THP-1	120	16
Ramos	230	16
spleen	120	70

→ higher E3 ligase levels (E_0) and longer protein half-life ($t_{½,P}$) lead to greater deg.

→ baseline POI levels (P_0) are of minor concern here



→ multiple compounds were tested in different cell lines

➢ Degradation in new **cell types** can be predicted from **physiological** parameters

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[5] data: *Mathieson et al. (2018) Nat. Commun.* DOI: 10.1038/s41467-018-03106-1
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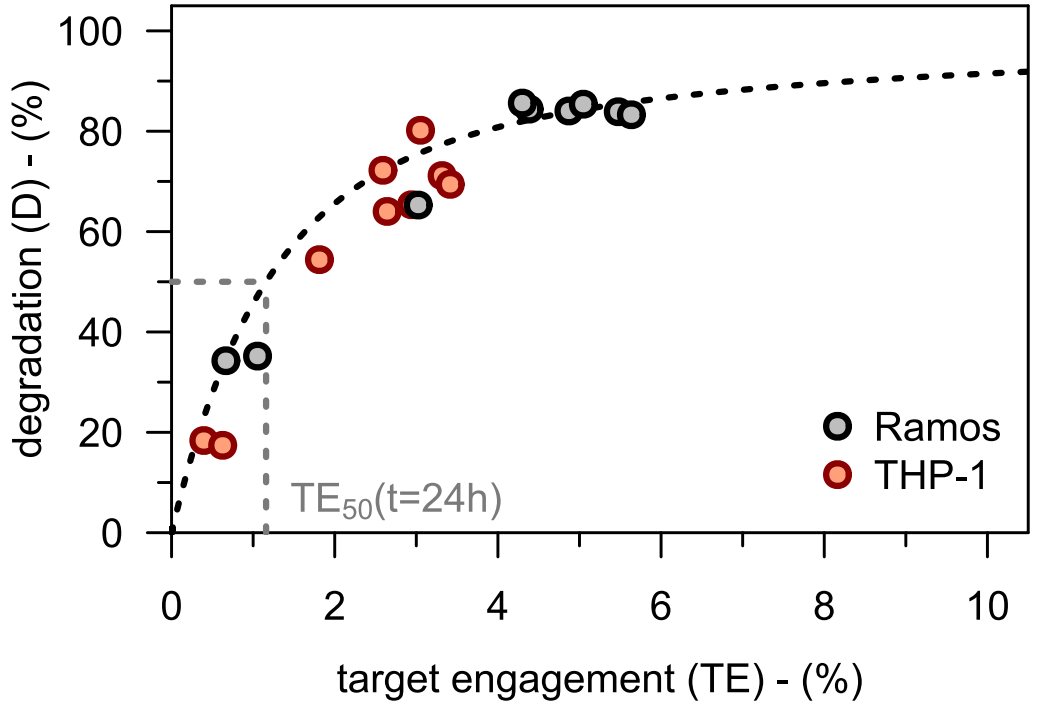
II) *Model-Informed Optimization of PROTACs*

Link target engagement (pillar II) to target degradation (pillar III) [3,6]

→ there are diminishing returns to increasing TE (hyperbolic relation)

→ optimizing affinities might not always be sufficient (but here it is)

→ increasing drug conc. only increases TE up to a set max. value (hook effect)



→ max. degradation is plotted vs. max. target engagement for different compounds

➢ PROTACs require **little** target **engagement** due to their **catalytic** MOA

[3.] data: *Zorba et al. (2018) Proc. Natl. Acad. Sci. USA* DOI: 10.1073/pnas.1803662115
[6.] data: *Bradshaw et al. (2015) Nat. Chem. Biol.* DOI: 10.1038/nchembio.1817

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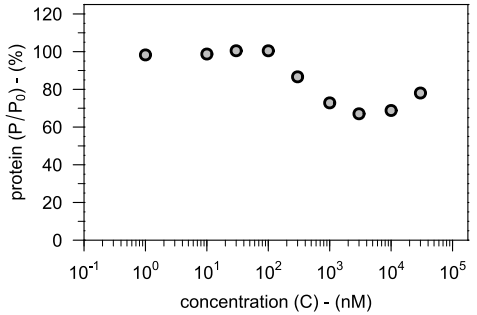
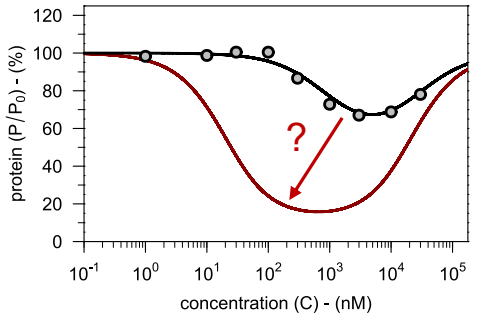


hook model

- $D_{\max}$
- DC_{50}
- $DC_{\max}$

II) Model-Informed Optimization of PROTACs

- How to increase degradation?



k_{cat} model

- $K_{\mathrm{D,P}}$ ↓
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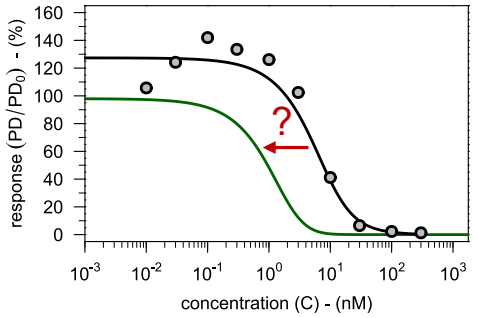
- α ↑

III) Deriving a Target Value for Degradation

- How much degradation is necessary?



PD model

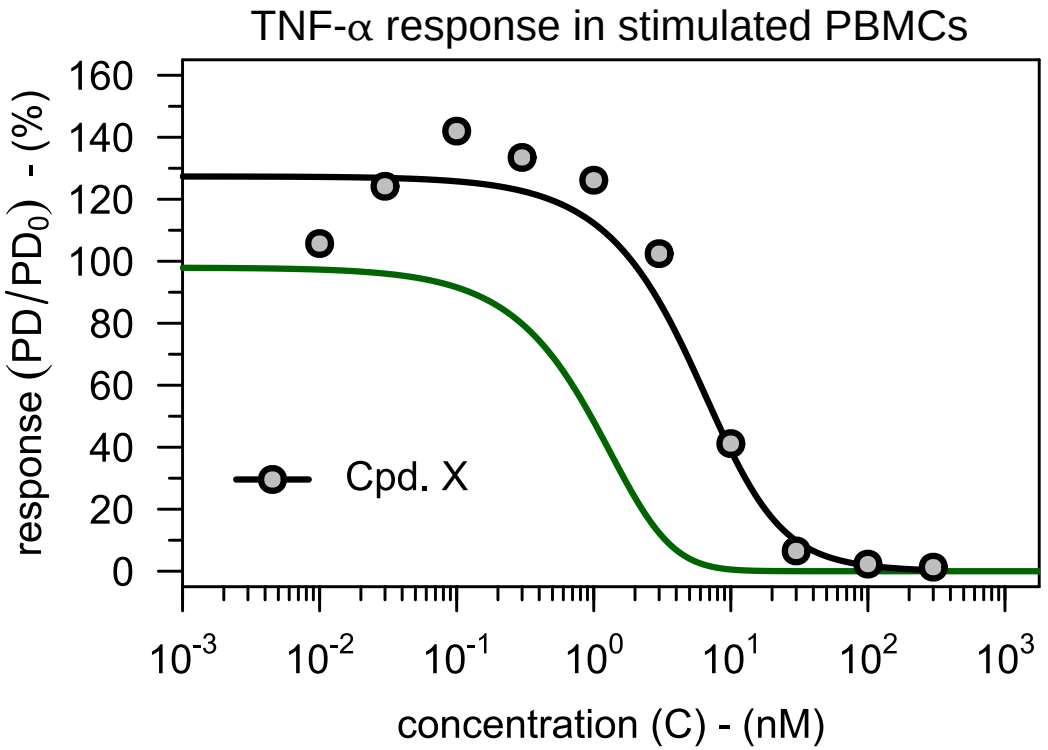
- $D_{\max}$ ↑
- DC_{50} ↓
- $DC_{\max}$ ↑

III) *Deriving a Target Value for Degradation*

Translate degradation to a downstream pharmacodynamic effect [8]

	Cpd. X
D_{max} (%)	53
DC_{50} (nM)	15
DC_{max} (nM)	NA

→ more degradation means less TNF-α response



1) the *PD model* [2] relates the observed PD response to protein degradation

➢ The most **relevant** PD **effects** are located **downstream** of protein degradation

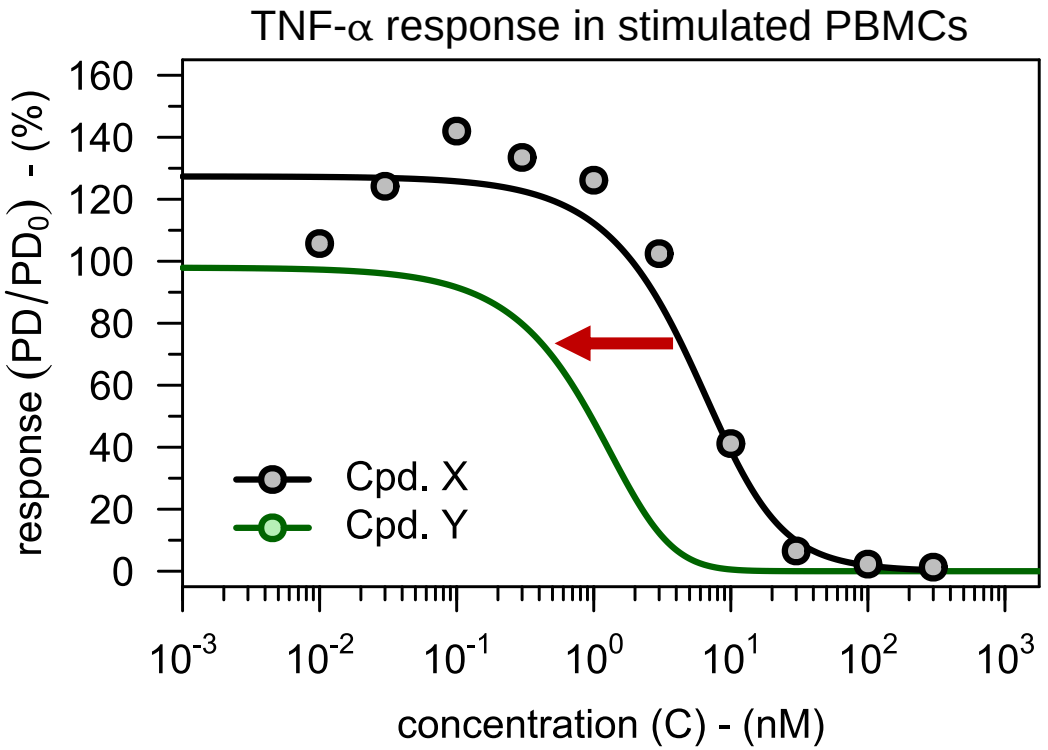
[2] *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195
[8] data: *Mares et al. (2020) Commun. Biol.* DOI: 10.1038/s42003-020-0868-6

III) *Deriving a Target Value for Degradation*

Translate degradation to a downstream pharmacodynamic effect [8]

	Cpd. X	Cpd. Y
D_{max} (%)	53 →	+20
DC_{50} (nM)	15 →	×3
DC_{max} (nM)	NA	NA

→ extent of degradation for desired PD response



1) the *PD model* [2] relates the observed PD response to protein degradation

2) it allows to translate target values for PD response to the level of degradation

➢ The *PD model* [2] **links** those **downstream** effects directly **to** target protein **degradation**

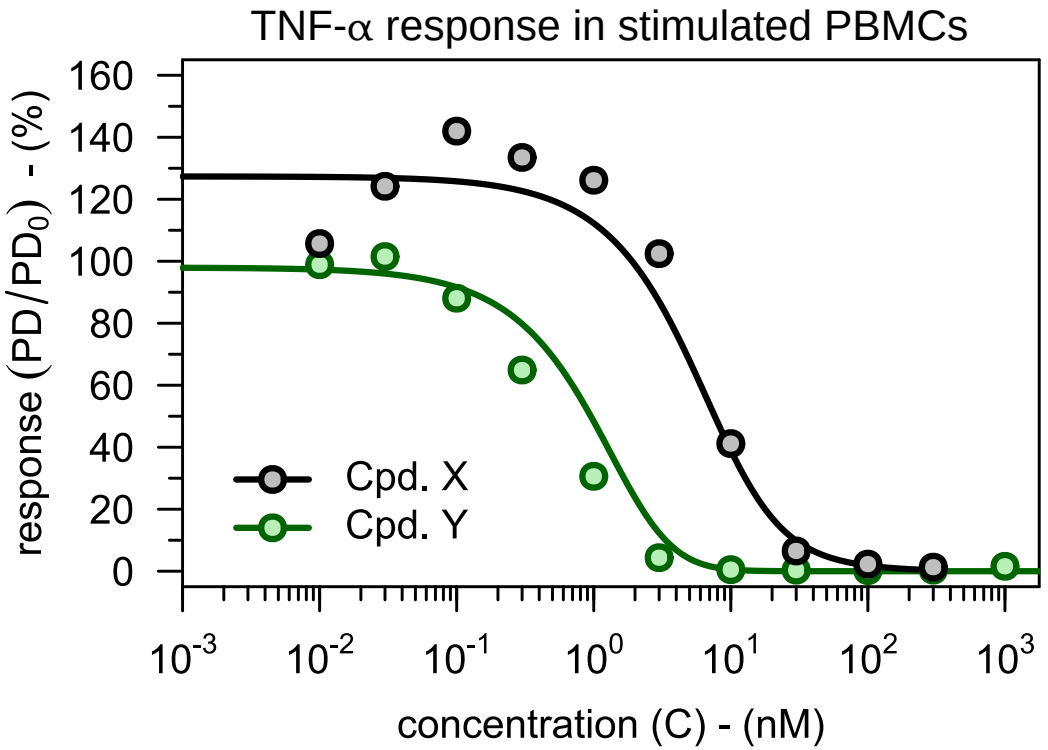
[2] *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195
[8] data: *Mares et al. (2020) Commun. Biol.* DOI: 10.1038/s42003-020-0868-6

III) *Deriving a Target Value for Degradation*

Translate degradation to a downstream pharmacodynamic effect [8]

	Cpd. X	Cpd. Y
D_{max} (%)	53	74
DC_{50} (nM)	15	5
DC_{max} (nM)	NA	142

→ for both cpds., 13% deg. gives 50% TNF-α inhib.



1) the *PD model* [2] relates the observed PD response to protein degradation

2) it allows to translate target values for PD response to the level of degradation

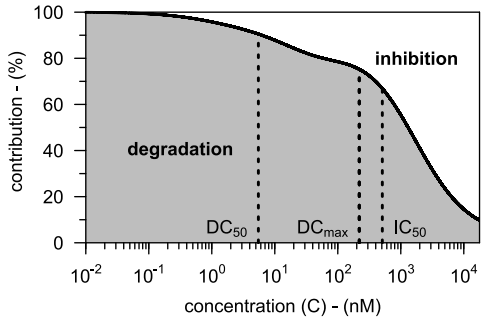
3) running the TNF-α assay on the optimized Cpd. Y confirms the predictions

➢ Establishing such a mechanistic model **validates** the degraded **protein** as a **target**

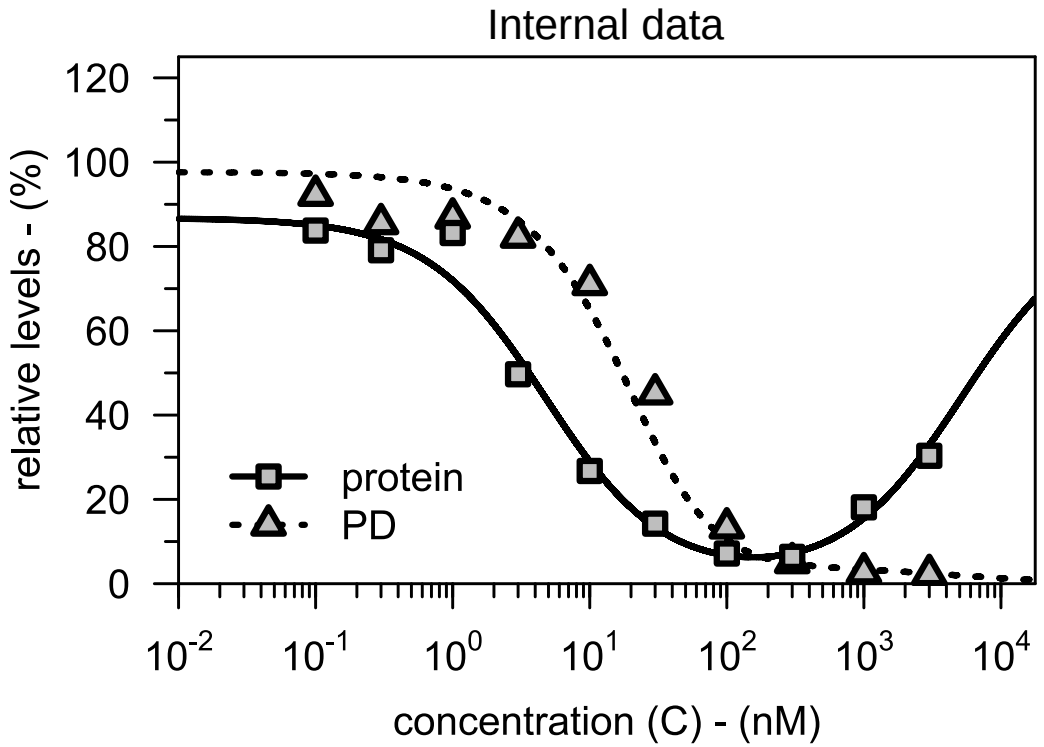
[8] data: *Mares et al. (2020) Commun. Biol.* DOI: 10.1038/s42003-020-0868-6

III) *Deriving a Target Value for Degradation*

Consider that PROTACs act both as degraders and as inhibitors



→ at high conc., effects are driven by inhibition



→ the PD model was fitted to these data and to two other profiles (not shown)

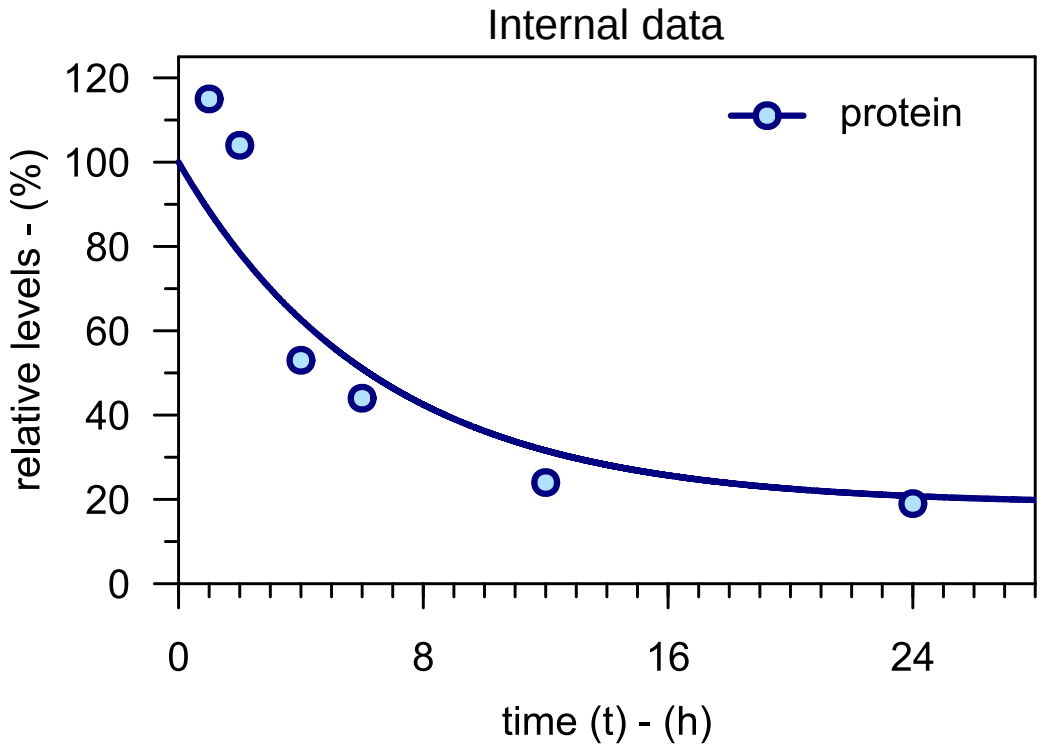
➢ Inhibition by the PROTAC **compensates** for the **hook** effect in degradation

III) *Deriving a Target Value for Degradation*

Consider that PROTACs act both as degraders and as inhibitors [5]

	PROTAC
D_{24h} (%)	79
$t_{½,P}$ (h)	24

→ *A priori*, only a single time-point is known



1) the time-course of deg. is predicted from the protein's baseline half-life

➢ The **protein's** baseline **half-life** determines the **time-course** of degradation

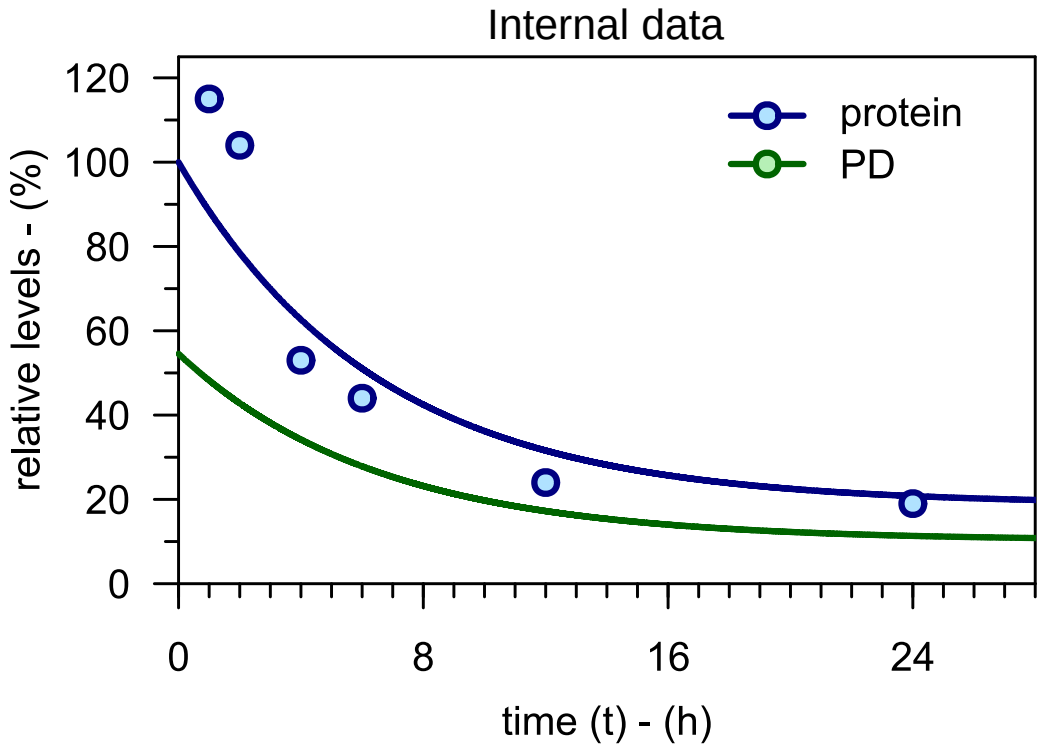
[5] data: *Mathieson et al. (2018) Nat. Commun.* DOI: 10.1038/s41467-018-03106-1

III) *Deriving a Target Value for Degradation*

Consider that PROTACs act both as degraders and as inhibitors [5]

	PROTAC	control
D_{24h} (%)	79	NA
$t_{½,P}$ (h)	24	NA
IC_{50} (nM)	---	120

→ more degradation means less PD response



1) the time-course of deg. is predicted from the protein's baseline half-life

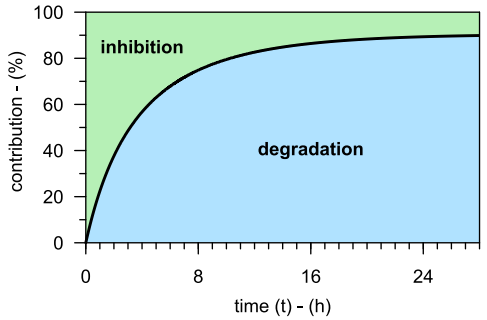
2) a degradation-incompetent control cpd. is used to estimate inhibitory potency

➢ Inhibition might **obscure** the **relationship** between degradation and the PD response

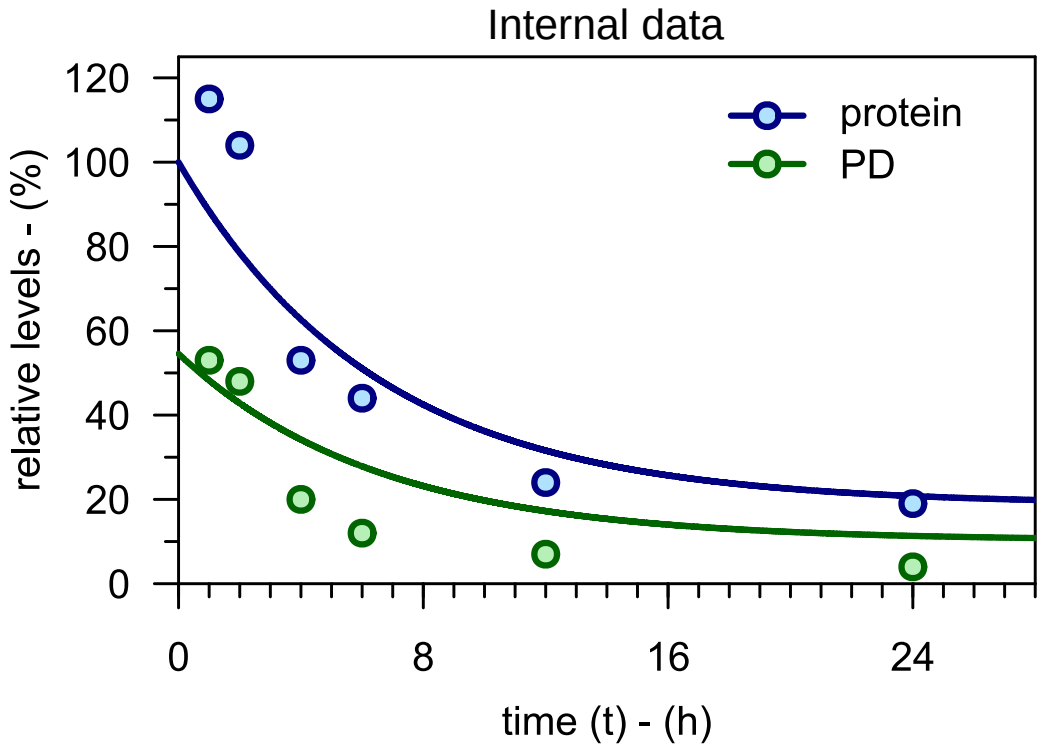
[5] data: *Mathieson et al. (2018) Nat. Commun.* DOI: 10.1038/s41467-018-03106-1

III) *Deriving a Target Value for Degradation*

Consider that PROTACs act both as degraders and as inhibitors [5]



→ early on, effects are driven by inhibition



1) the time-course of deg. is predicted from the protein's baseline half-life

2) a degradation-incompetent control cpd. is used to estimate inhibitory potency

3) the time-course of the PD response is predicted for the active PROTAC

➢ PROTACs that act as both **degraders & inhibitors** might feature a more **rapid** onset of action

[5] data: *Mathieson et al. (2018) Nat. Commun.* DOI: 10.1038/s41467-018-03106-1

Take-Home Messages

Three distinct applications for pharmacodynamic modeling of in vitro data

I) Assessing PROTACs as Degraders

- The ***hook model*** [2] captures how degradation depends on:
  -) PROTAC concentration → hook effect
  -) incubation time → extrapolation to steady state profile

II) Model-Informed Optimization of PROTACs

- The $\boldsymbol{k_{cat}}$ ***model*** [2] predicts degradation from biochemical parameters
  -) to optimize a compound, increase its three binding affinities
  -) consider expression levels of E3 ligase and protein half-life when translating *in vitro* data

III) Deriving a Target Value for Degradation

- The $\boldsymbol{PD}$ ***model*** [2] translates degradation to a downstream effect
  -) define a target value for the PD effect and translate that to a target value for degradation
  -) inhibitory activity of PROTACs allows for rapid onset of action & compensates for hook effect

[2.] *Haid & Reichel (2023) Pharmaceutics* DOI: 10.3390/pharmaceutics15010195

To be Continued …

Going in vivo, implications for drug discovery & real-world case studies

1) Translation from biochemical to cellular level

   - How to increase degradation potency?

2) Translation from cellular level to animal model

   - Which compounds to take *in vivo*?

3) Translation from animal model to human

   - What is the relevant dose in humans?

Link: https://tinyurl.com/yby3k25w

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Discussion

What do you think about all of this?