

Modelling framework to evaluate anti-viral PROTACs

Yi-Han Chien¹, You-Ting Chen¹, Judith Röske², Kaixuan Zhang², Graham P. Marsh³, Sebenzile K. Myeni⁴, Hannah J. Maple³, Alex Moloney³, Marjolein Kikkert⁴, Rolf Hilgenfeld^{2,5}, Mark Brönstrup^{1,6}, Katharina Rox^{1,6}

¹Department of Chemical Biology (CBIO), Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany

²Institute of Molecular Medicine, University of Lübeck, Lübeck, Germany

³Bio-Techne (Tocris), Bristol, United Kingdom

⁴Leiden University Medical Centre, Leiden, the Netherlands

⁵German Center for Infection Research (DZIF), Partner site Lübeck-Borstel-Riems, Germany

⁶German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig, Germany

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Background

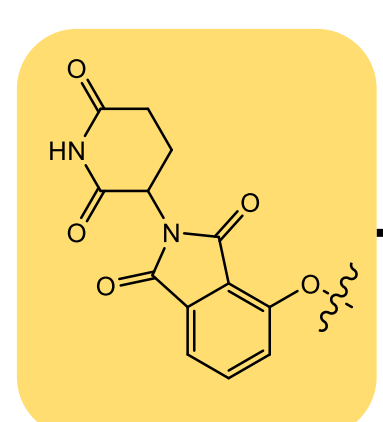
Proteolysis-targeting chimeras (PROTAC) are novel chemical concepts taking advantage of the proteasome ubiquitination system to degrade the protein of interest (POI). Compared to oncological PROTACs, which have already entered clinical trials¹, the development of PROTACs against infectious diseases has only recently accelerated². When designing PROTACs, potent inhibitors of the targeted protein are commonly deployed as the POI binding ligand. With the viral protein as the main target for anti-viral drugs, covalent inhibitors (both reversible and irreversible) are increasingly explored due to less toxicity concerns. Thus, covalent inhibitors speak for using them as the POI binding ligand in anti-viral PROTAC design³. Currently, PROTACs are mostly equipped with a reversible non-covalent POI binding ligand. To date, the consequences of a more robust or irreversible binding to the target protein with respect to degradation efficiency remain obscure.

Method

- Extension of the current mechanistic PD model of non-covalent PROTAC⁴ to assess covalent binding anti-viral PROTACs
- Prediction of the in vivo anti-viral efficacy by linking the PK/PD model to a viral dynamics model
- Validation of the model with in-house in vitro and in vivo efficacy results of PROTAC-1
- Development of modeling framework and Simulation using the *nlmixr2*¹² and *rxode2*^{13,14} packages in R

PROTACs employing POI inhibitors of difference binding mechanisms

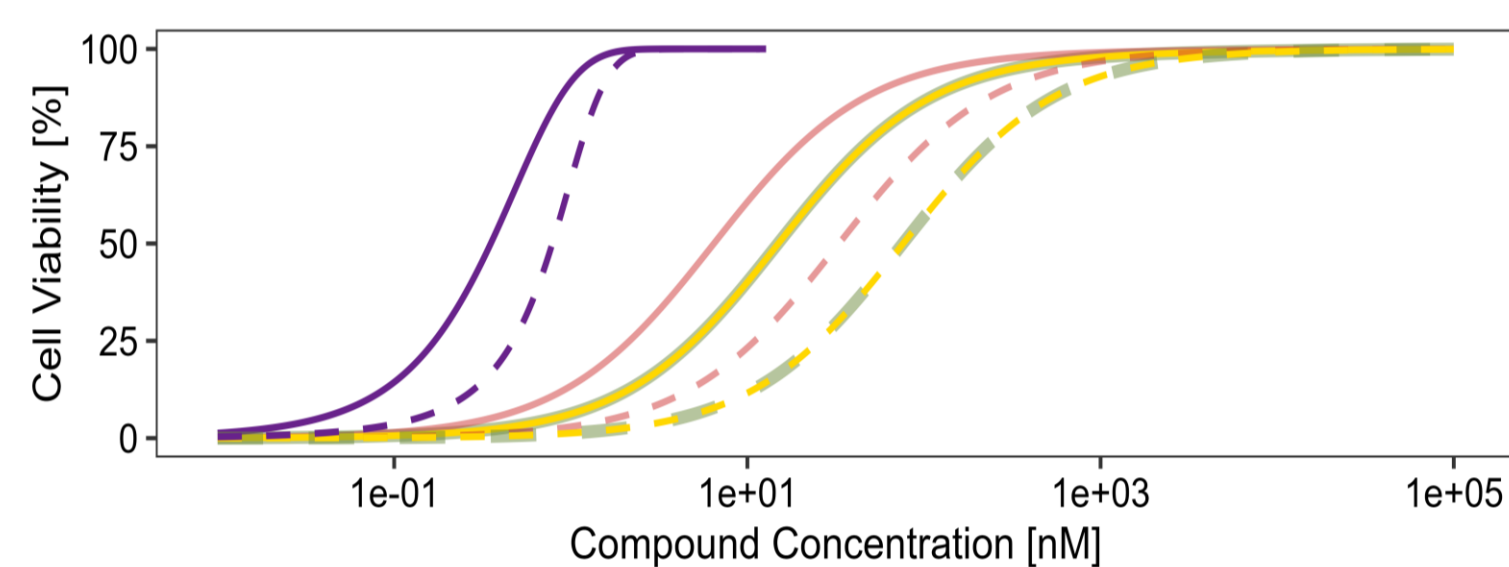
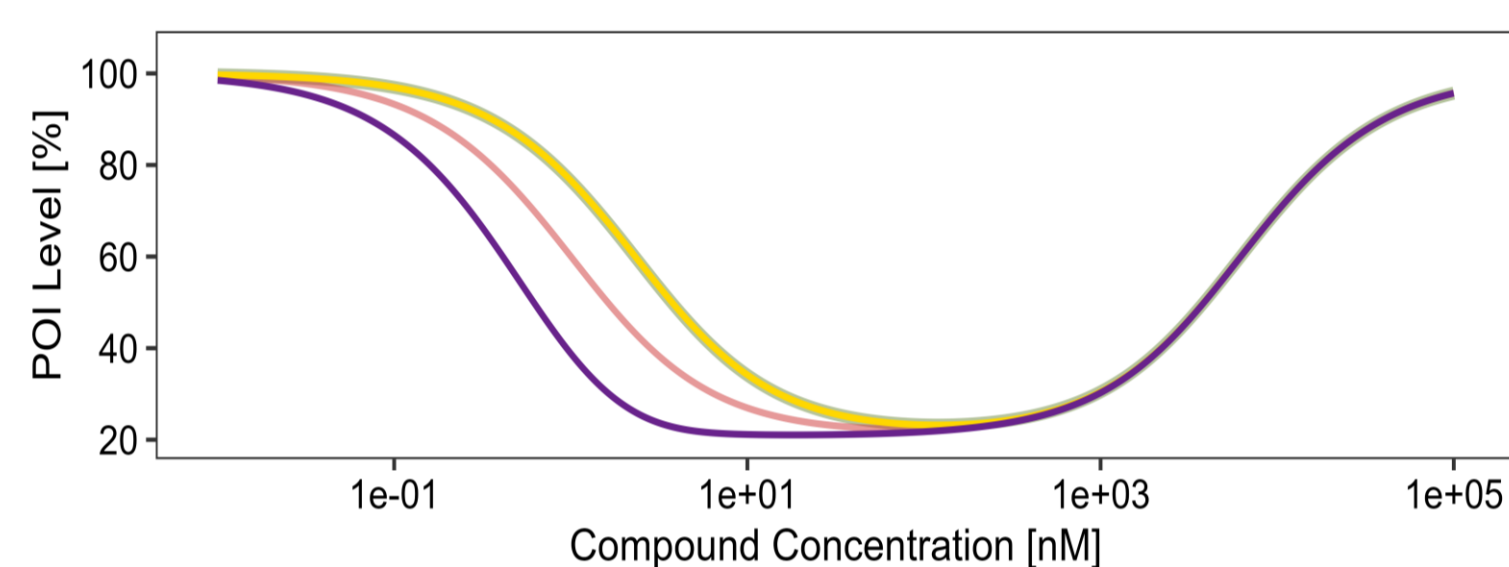
Construction of hypothetical PROTACs



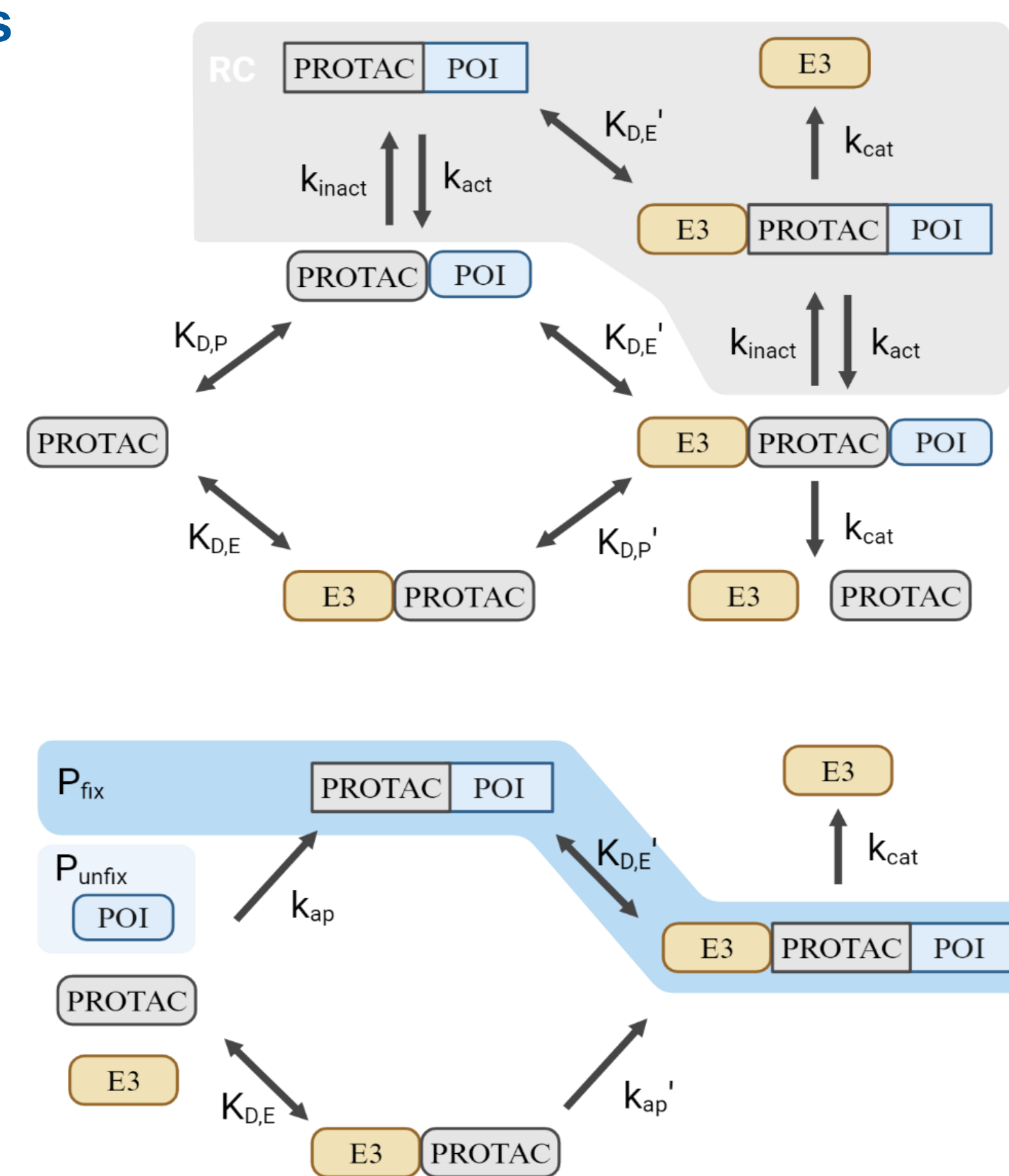
- Reversible Covalent (RC):**
 - Nirmatrelvir & RAY1216
- Reversible Non-Covalent (RN):**
 - Ensitrelvir
- Irreversible Covalent (IR):**
 - N3

E3-ligase Recruiter

Environmental Parameter		M ^{pro} Inhibitor	
E_0	[nM]	66.3 (Vero E6)	
$t_{1/2,P}$	[h]	15.8	
k_{cat}	[1/h]	4.6 [4]	
PROTAC Affinity Parameter			
$K_{D,E}$	[nM]	1700 [5]	
α		1	
Nirmatrelvir	K_i [nM]	3.67 [6]	
RAY1216	K_i [nM]	8.47 [6]	
Ensitrelvir	$K_{D,P}$ [nM]	8.43 [7]	
N3	k_{ap} [M ⁻¹ s ⁻¹]	11300 [8]	

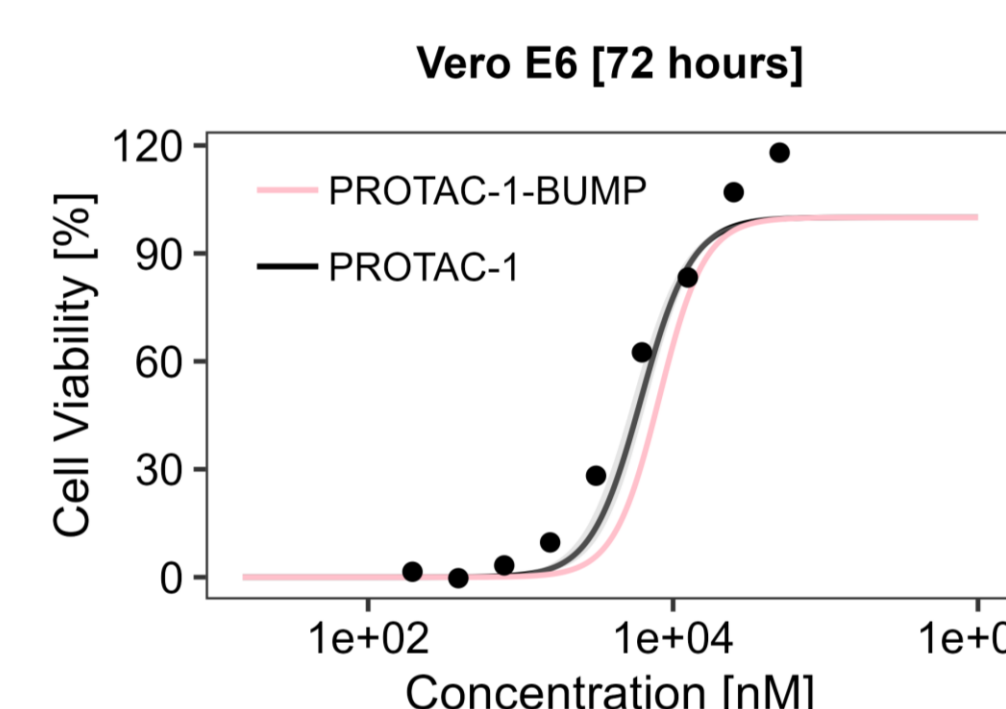
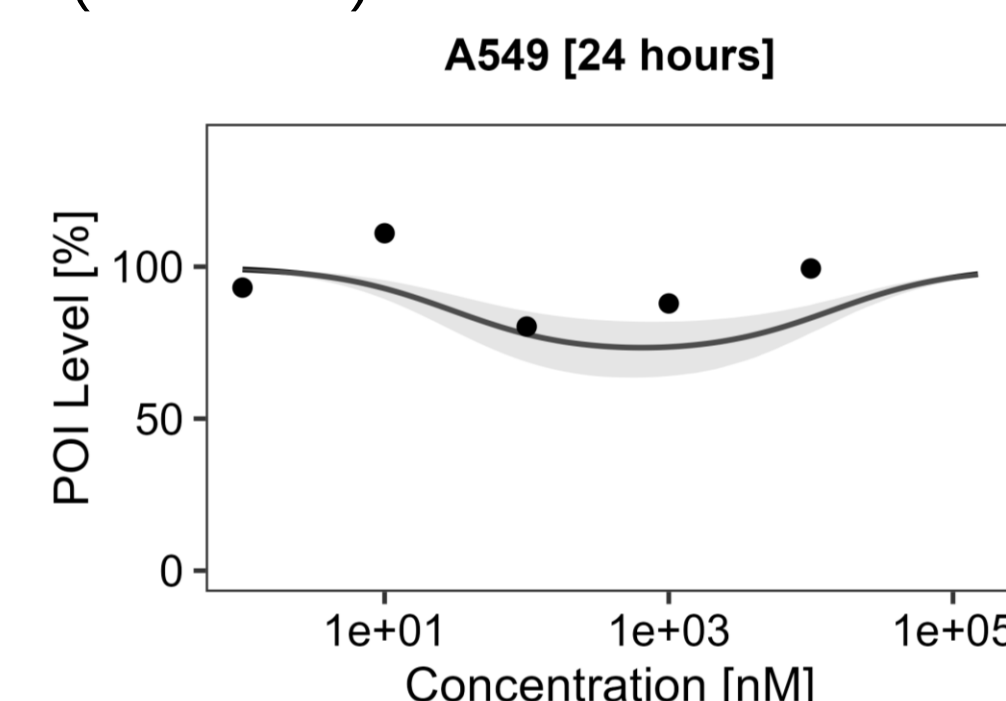


$$\frac{EC_{50,RN,PROTAC}}{EC_{50,RN,Bump}} \approx \frac{EC_{50,RC,PROTAC}}{EC_{50,RC,Bump}} < \frac{EC_{50,IR,PROTAC}}{EC_{50,IR,Bump}}$$



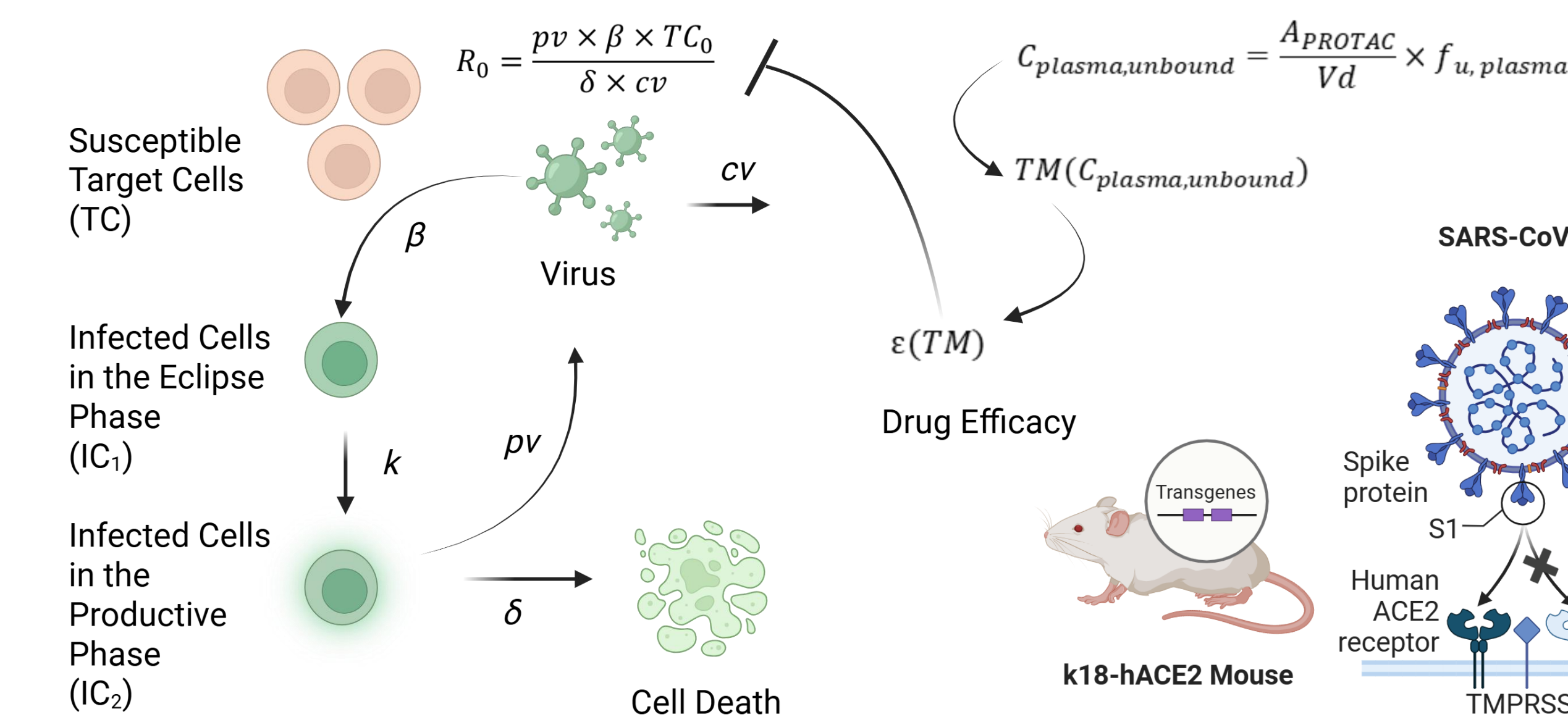
PROTAC-1

- 13b-K⁹ as warhead (RC-inhibitor of M^{pro})
- Affinity parameters ($K_{D,E}$, K_i , and α) estimated by biophysical methods (MST/SPR)

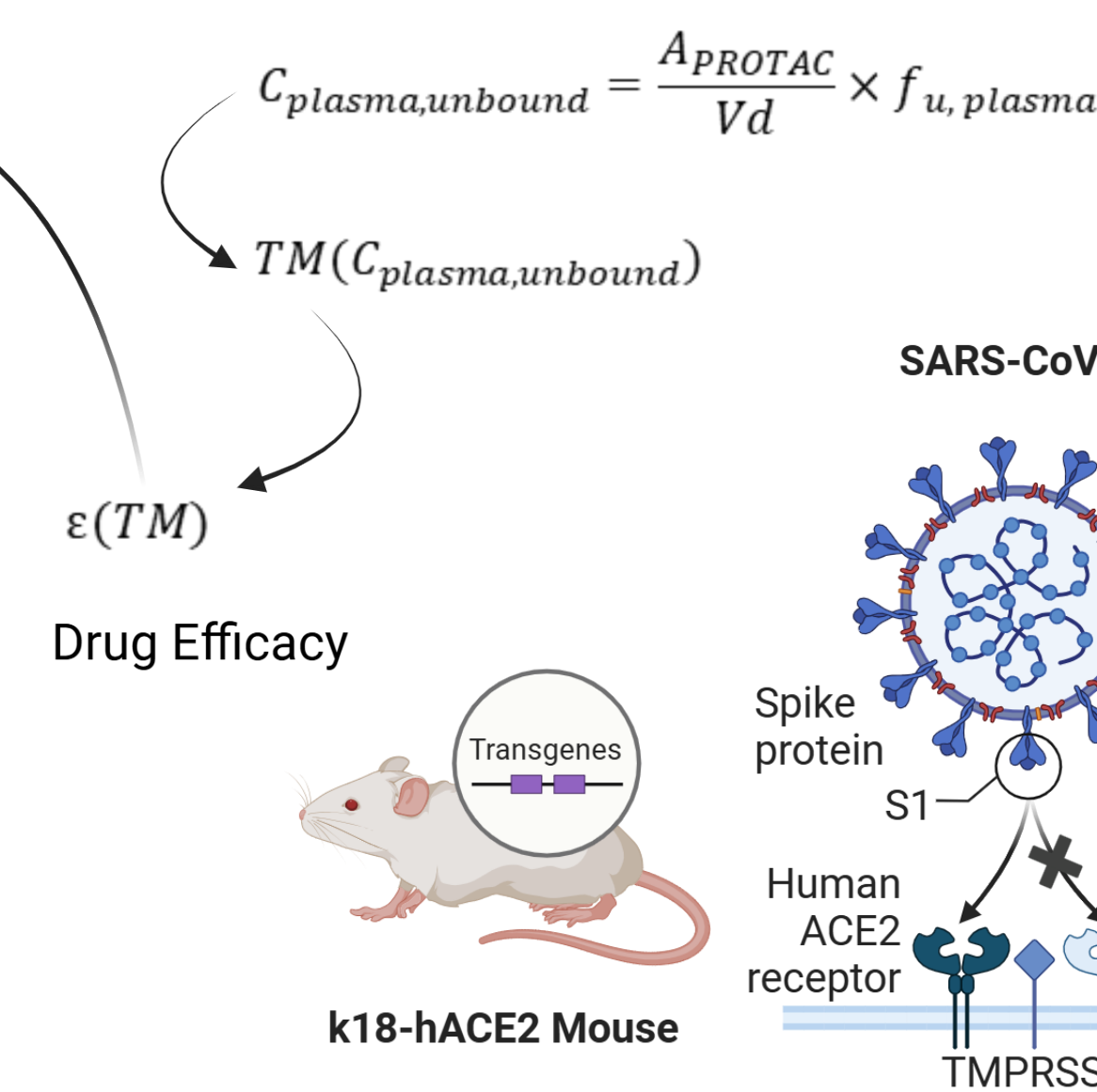


Construction of modelling framework to inform anti-viral effect

Viral Dynamic Model



PK/PD Model



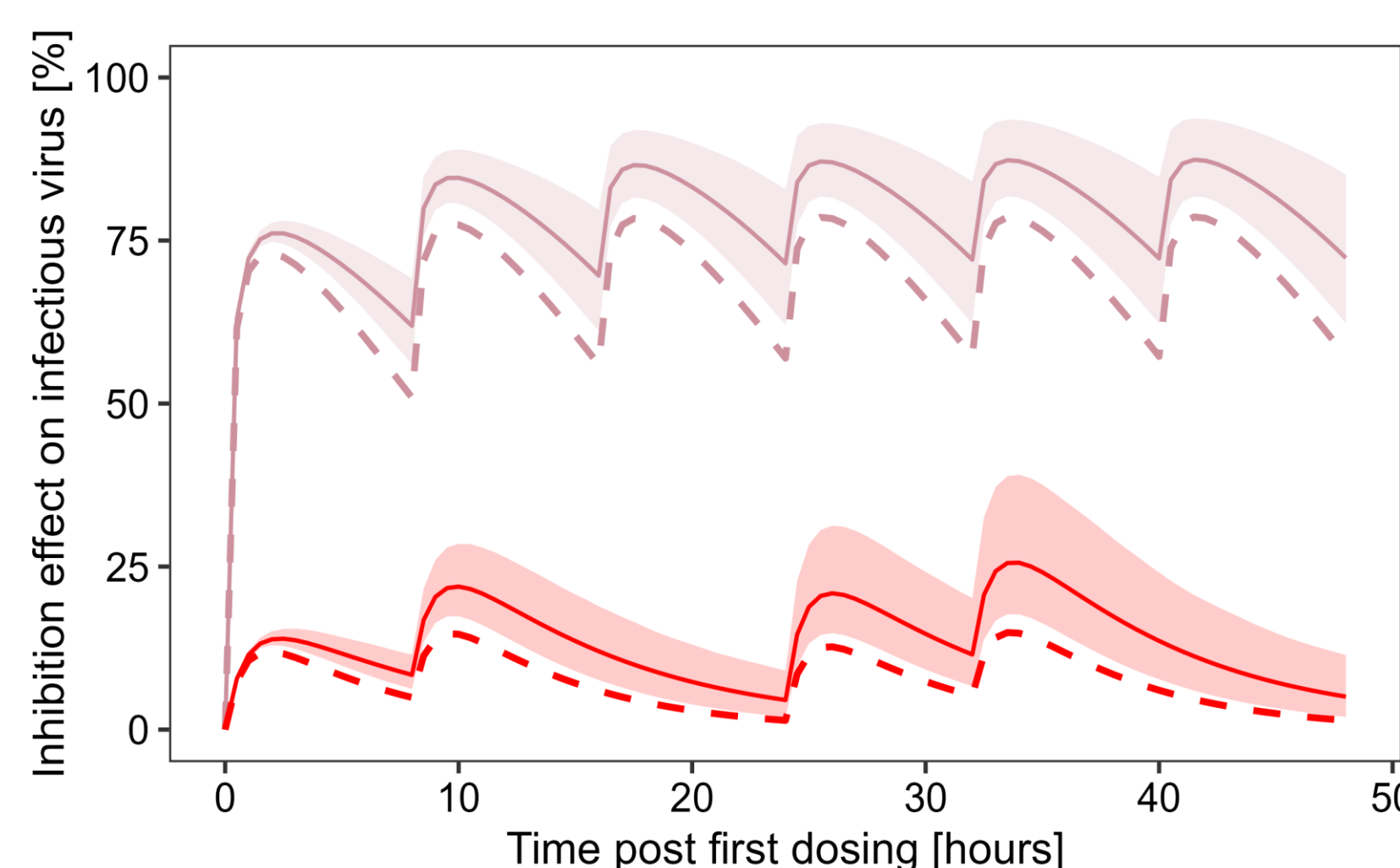
Current Study with k18-hACE2 mice			Gonçalves et al. with Covid-19 patients		
Parameter	Value	(95% CI) Unit	Value	(95% CI) Unit	Unit
p_v	0.012	(0.0084, 0.017) PFU·cell ⁻¹ ·day ⁻¹	22.7	(0, 59.6) copies·cell ⁻¹ ·day ⁻¹	
c_v	5.45	(4.09, 7.27) 1/day	10	(Fix) 1/day	
δ	1.44	(1.27, 1.62) 1/day	0.60	(0.22, 0.97) 1/day	
R_0	9.69	(7.86, 11.9) Cells	8.60	(1.90, 17.6) Cells	
k	3	(Fix) 1/day	3	(Fix) 1/day	
TC_0	1.67×10^9	(Fix) cells/g	1.33×10^5	(Fix) cells/mL	

- Parameters are characterized based on published time course of lung viral load post SARS-CoV-2 infection (2×10^4 PFU) in k18-hACE2 mice¹⁰
- Plaque forming unit per gram lung tissue as model output
- Parameters are comparable to those estimated for human

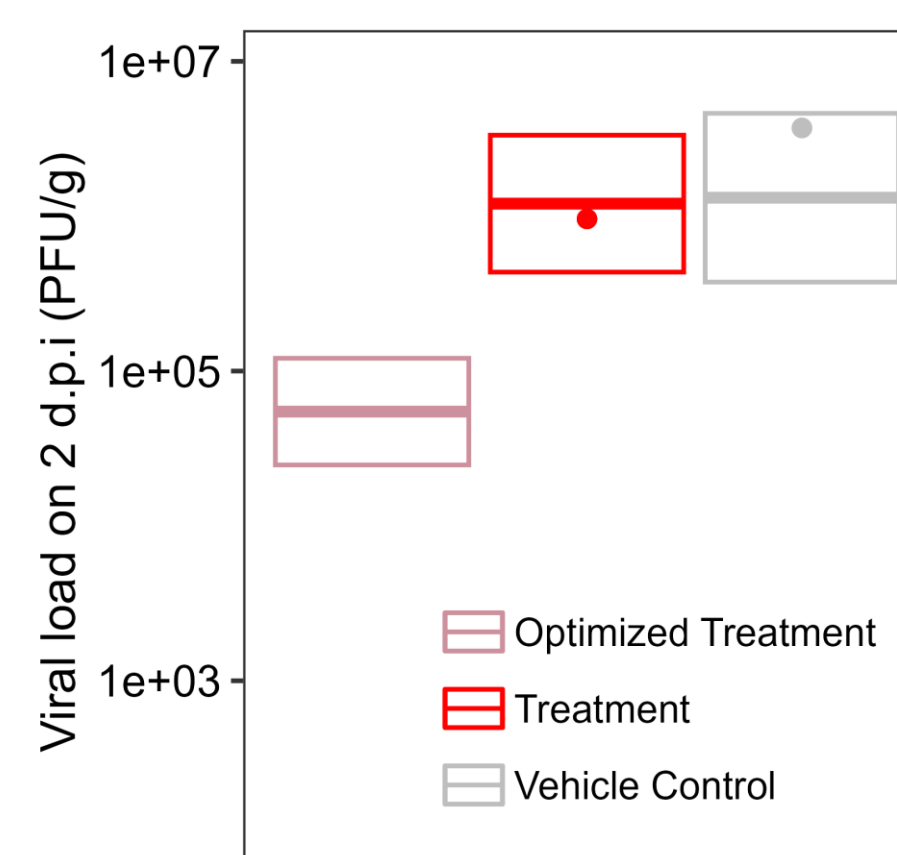
Model informed in vivo anti-viral effect

in vivo efficacy study was conducted for PROTAC-1

- K18-hACE2 mouse model
- Two-day treatment initiated concurrently with SARS-CoV-2 infection (1×10^4 PFU)
- Treatment regimen: 50 mg/kg IP. twice per day at t = 0 and 8 hours



- In vitro EC_{50} of the warhead compound used to inform the PD model: 13b-K against SARS-CoV-2 infectious viral release in Calu3 cells⁹ ($EC_{50} = 2.4 \mu M$)



Viral load per gram lung tissue

- Model predictions support the experimental observations of no significant difference between the conducted treatment and vehicle groups
- Enables the identification of an optimized treatment regimen (100 mg/kg IP. three times per day at t = 0, 8, and 16 hours) predicted to yield a significant therapeutic effect (viral load reduction of more than 1 log₁₀ unit)

Summary and Perspectives

- The extended modeling framework allows for a preliminary evaluation of PROTAC design using existing inhibitors as binding warheads
- The **in vivo efficacy of an anti-viral PROTAC** can be **effectively predicted** using the current framework, offering valuable feedback on the compound selection process and on guiding the dosage regimen.
- The viral dynamics model, based on a humanized mouse model, provides insights for translating efficacy to humans.

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For more information:

Yi-Han Chien
yi-han.chien@helmholtz-hzi.de



LinkedIn



www.helmholtz-hzi.com

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