

Modelling framework to simulate phenotypic results of antiviral PROTACs

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Background

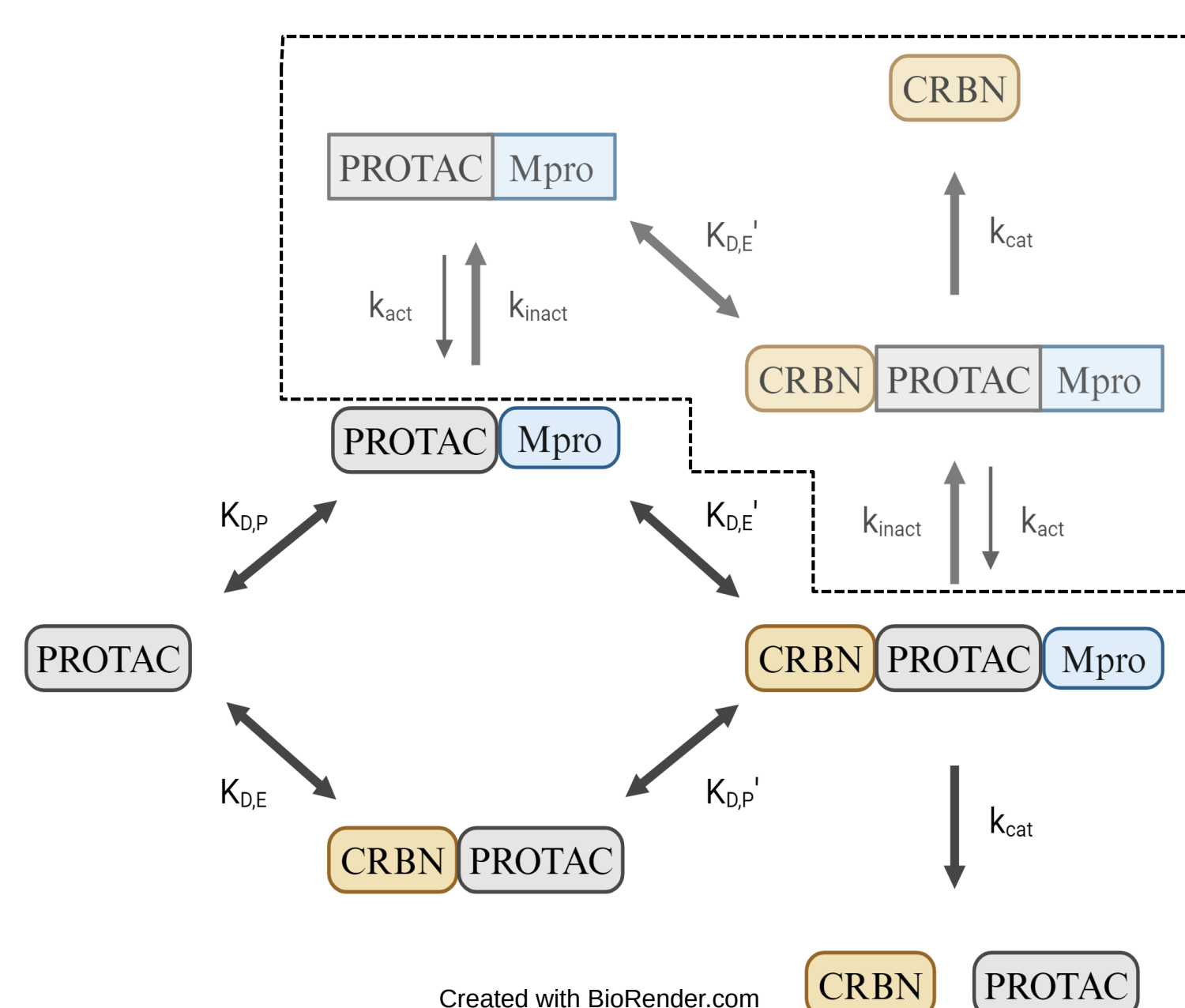
Proteolysis-targeting chimera (PROTAC) is a novel chemical concept taking advantage of the proteasome to degrade target proteins. Compared to PROTACs designed for oncology, which have already entered clinical trials¹, antiviral PROTACs are currently still in an early stage of drug development. Unlike oncological PROTACs, targeting primarily overexpressed endogenous proteins, antiviral PROTACs often target either host proteins, 'hijacked' to support viral infection, or viral proteins, presented during infection. To predict the degradation potential of antiviral PROTACs, we adjusted and fine-tuned current modelling frameworks to simulate efficacy of PROTACs compared to conventional small-molecule inhibitors.

Method

- A mechanistic PD model⁵ was deployed to predict the antiviral potential of a hypothetical PROTAC, employing 13b-K, a potent reversible covalent inhibitor of SARS-CoV-2 main protease (Mpro)^{2,3} and pomalidomide⁴ as warheads.
- The antiviral potential of PROTAC was assessed by comparing with Bump compound.
- A PBPK model of the warhead 13b-K was linked to the mechanistic model of PROTAC to get a first preview of in vivo efficacy.

Mechanistic Behavior of Reversible Covalent PROTAC

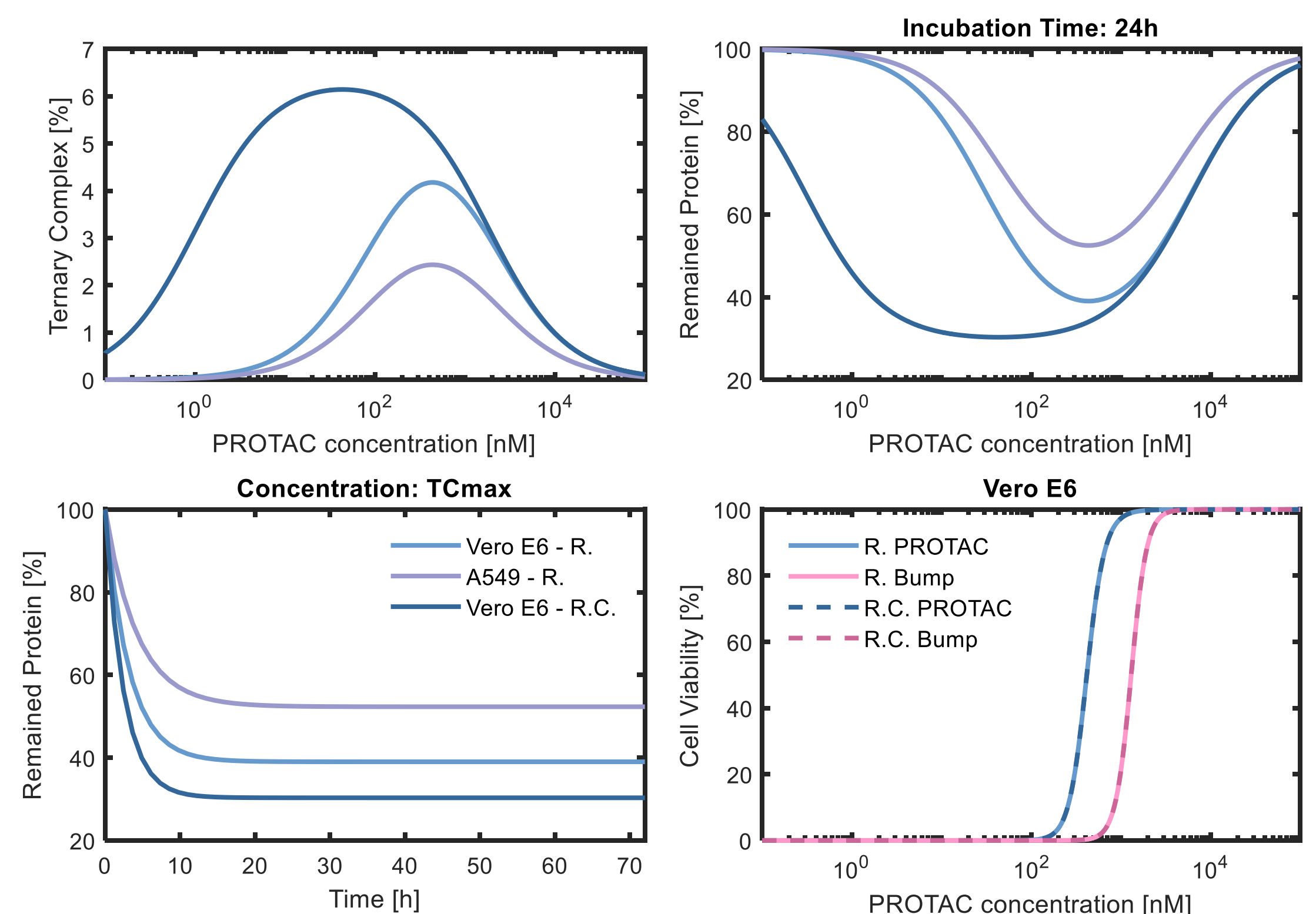
- Phenotypic result of reversible covalent PROTAC can be simulated by the mechanistic PD model of reversible PROTAC



Environmental Parameter			
CRBN	[nM]	Vero E6	A549
		117 *	67 *
t _{half-Mpro}	[h]	5.64	
k _{cat}	[1/h]	4.60 ⁵	
Compound Affinity			
K _{D,P}	[nM]	112	
K _{D,E}	[nM]	1,700 ⁴	
α _{Cooperative factor}		1	
k _{act} /k _{inact}		0.01	

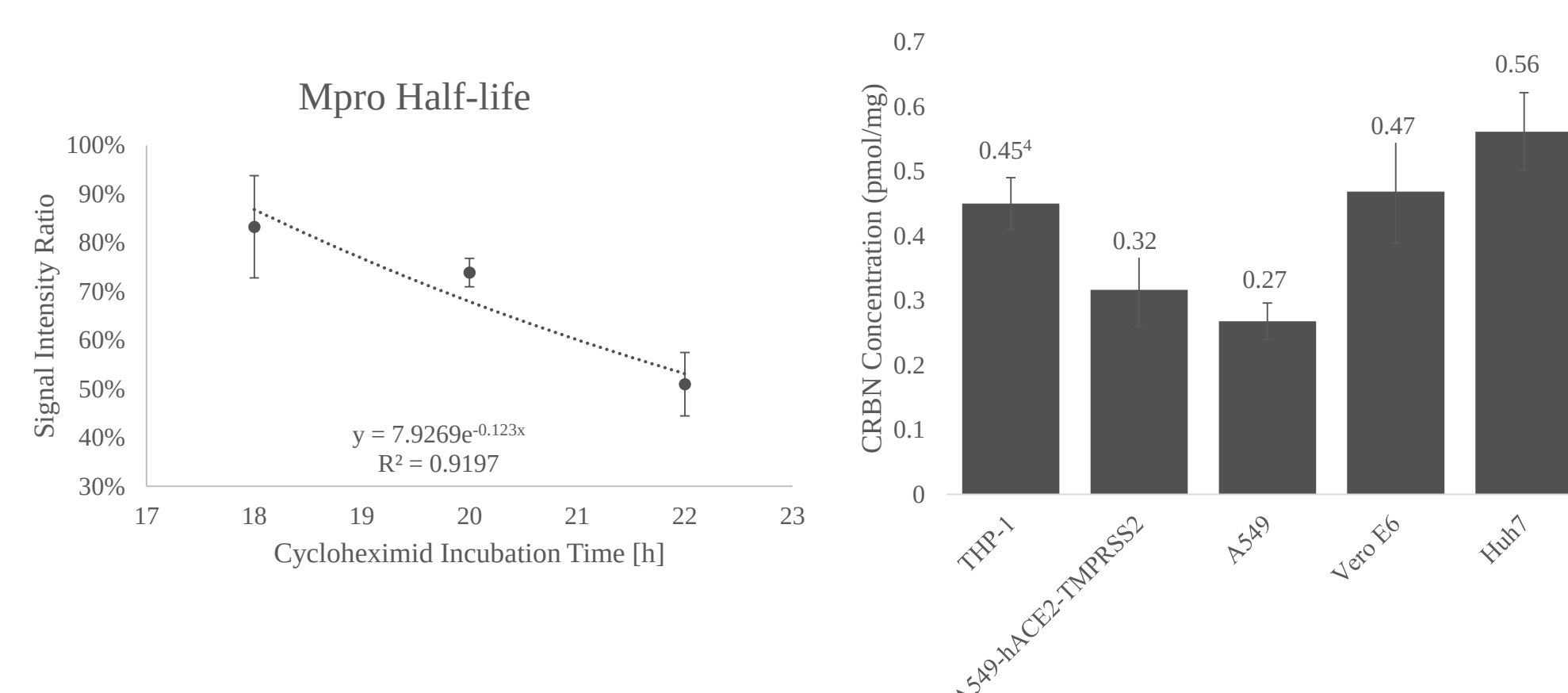
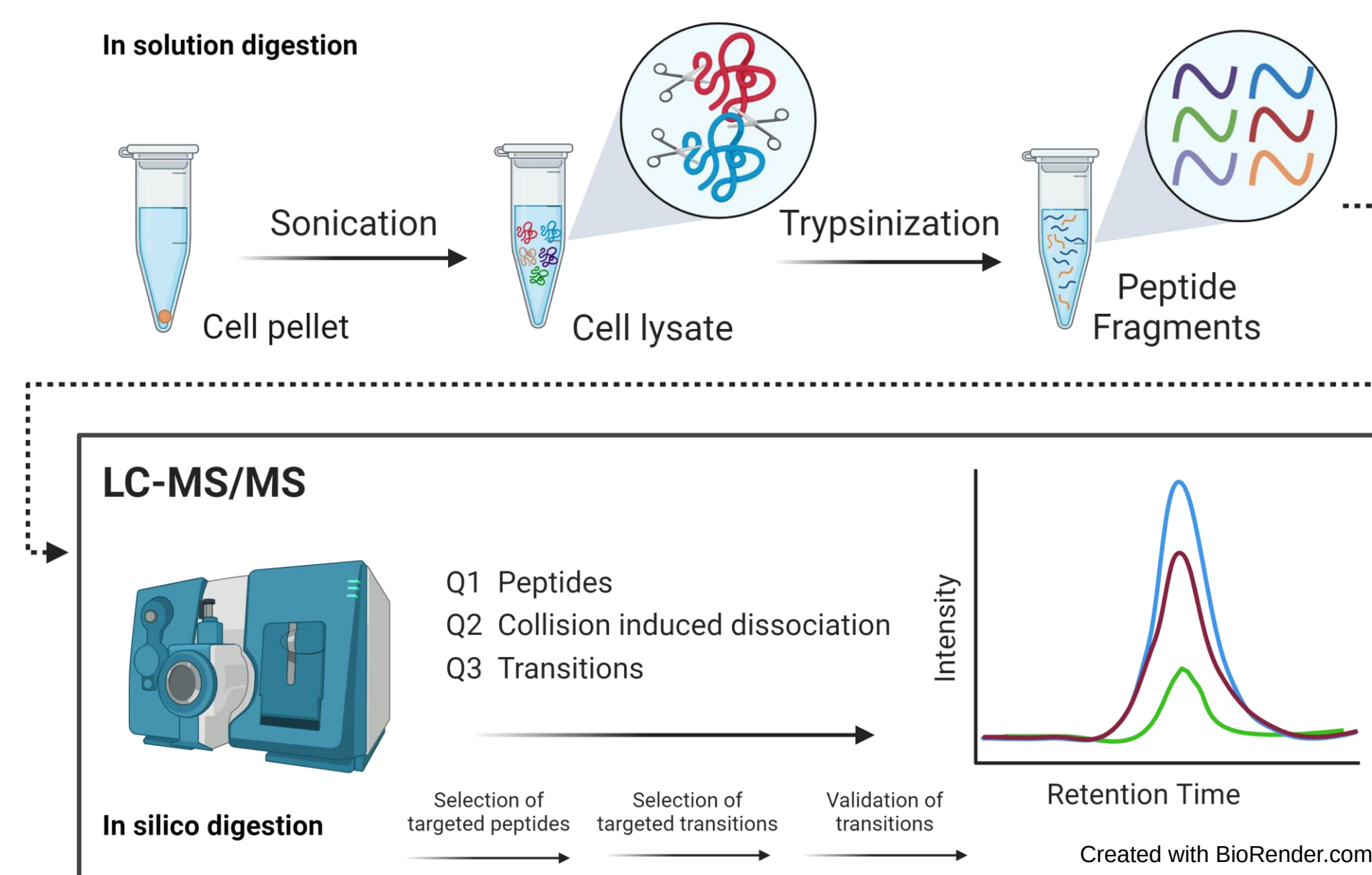
*Assume cell protein occupies 25% of cell volume

$$\frac{EC_{50, R. PROTAC}}{EC_{50, R. Bump}} = \frac{EC_{50, R.C. PROTAC}}{EC_{50, R.C. Bump}}$$

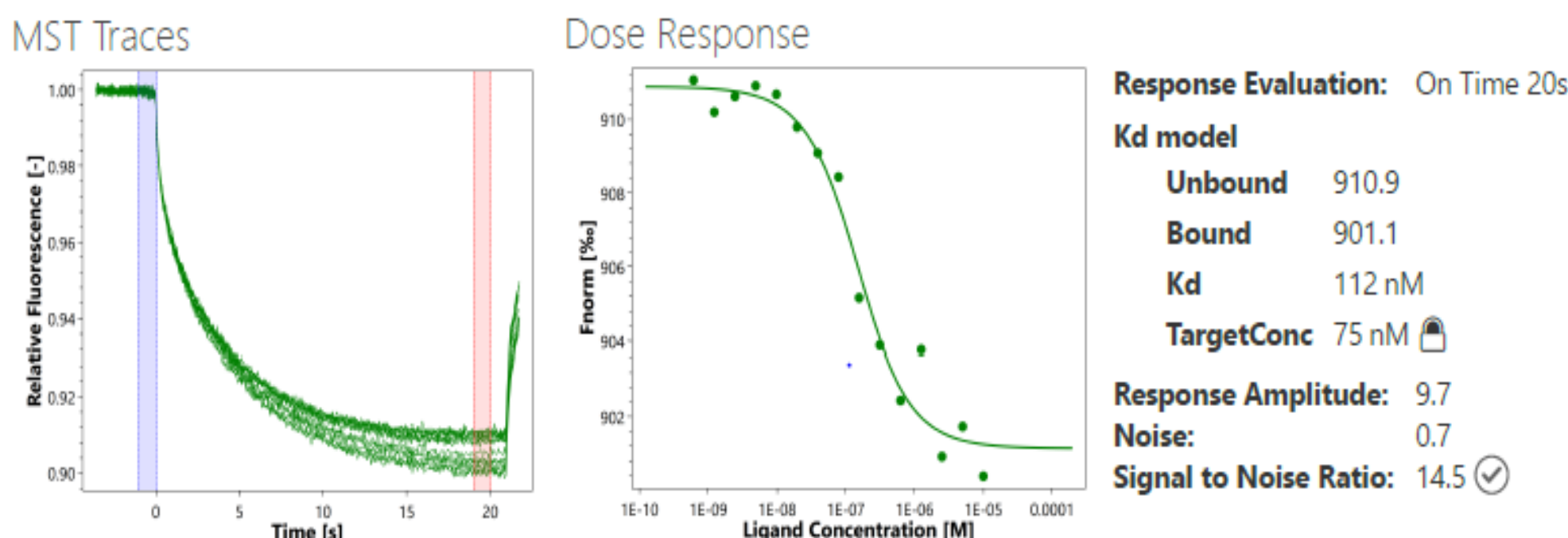


Parameter Estimation

Quantitative MRM based target proteomics

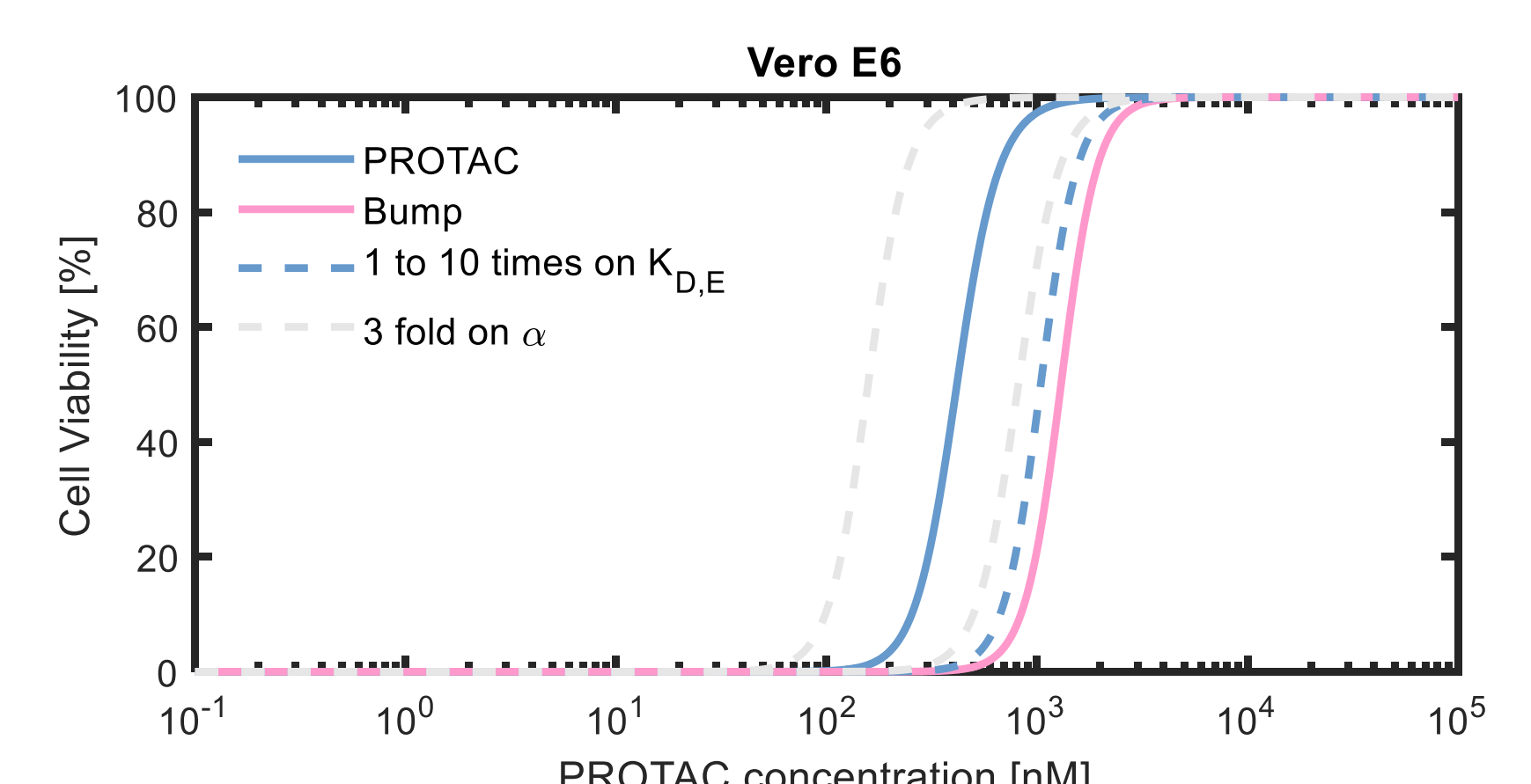
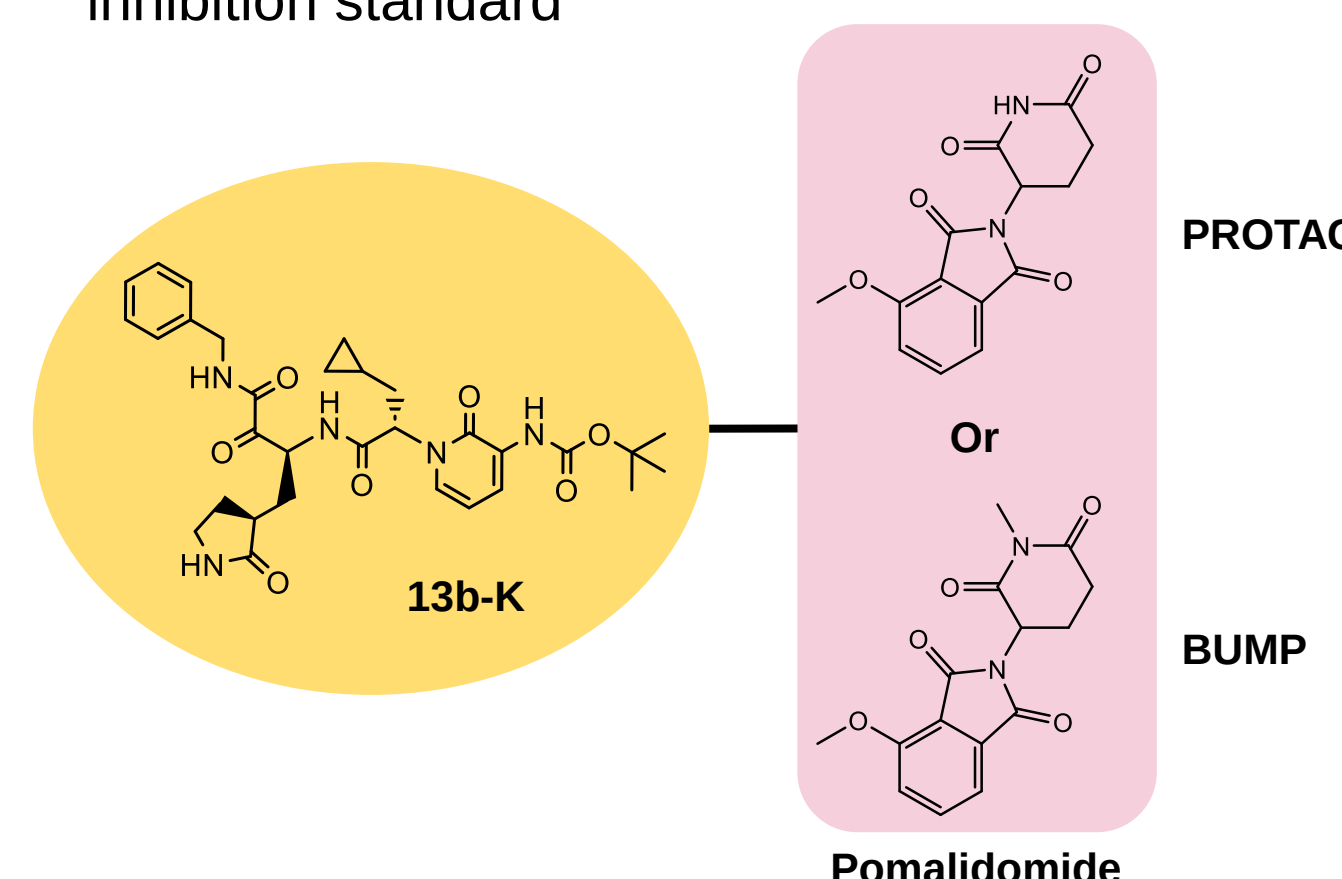


Microscale thermophoresis



Mechanistic Modelling – in vitro effect

- Cell viability as phenotypic result of antiviral effect
- Target Modulation represents the percentage of target protein being bound or degraded
- Bump compound with similar properties as inhibition standard



$$\text{Cell Viability [\%]} = \frac{Fl_{\text{treated}} - Fl_{\text{untreated control}}}{Fl_{\text{uninfected control}} - Fl_{\text{untreated control}}} \times 100\%$$

13b-K	SARS-CoV-2 Mpro	$\frac{EC_{50, PROTAC}}{EC_{50, Bump}}$
EC ₅₀ in Vero E6	TM50 in Vero E6	
1.30 μM ³ (1.16 – 1.55)	92% (91% – 93%)	0.29 (0.28 – 0.29)

Mechanistic PBPK Modelling – in vivo effect

- PBPK model of 13b-K was constructed based on in vivo PK data from mice and ADME assay results
- PD linked to intracellular unbound compound concentration in lung
- Elimination phase as observation period to evaluate in vivo degradation efficiency

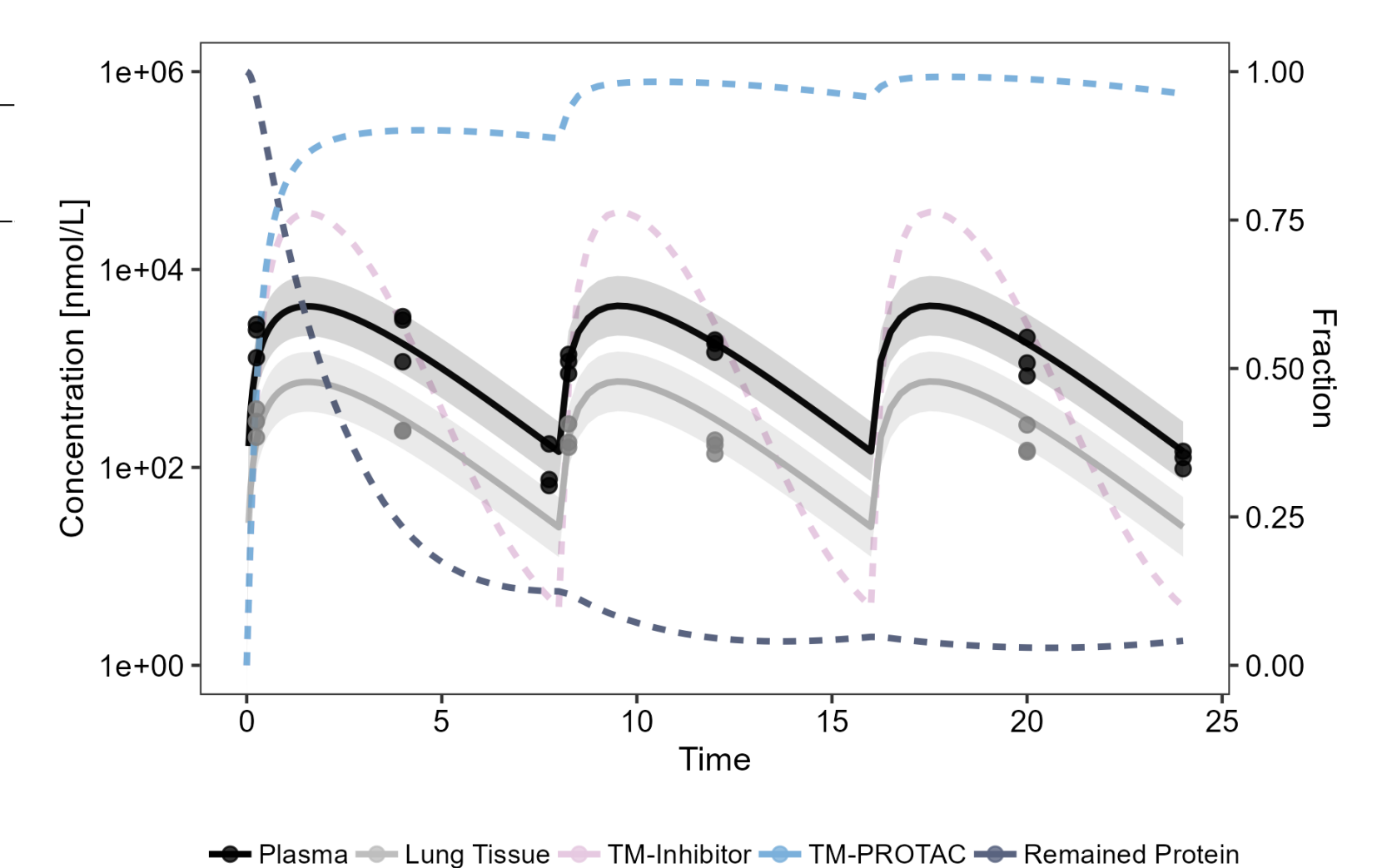
PBPK Parameter			
Molecular Weight [g/mol]	594		
logD _{7.4}	2.67	(Shake Flask)	
Plasma Protein Binding [%]	84.9	(RED Device)	
pKa	10.6 (Acid)	(Percepta®)	
Permeability [cm/min]	0.03 *	(0.23, PAMPA)	
Cl _{renal} [ml/min/kg]	34.0 *		
K(int/plasma)	0.13 *	(0.46, Schmitt)	
K(intra/plasma)	0.21 *	(2.01, R&R)	
k _{transit} [1/min]	0.02 *		
F [%]	56 *		

* Estimated (Ref)

PK-Sim

MoBi

50 mg/kg t.i.d. subcutaneous administration



Summary and Perspectives

- The proposed framework offers feedback on 1) PROTAC structure optimization, 2) in vitro assay optimization, and 3) in vivo study design.
- It enables an indirect validation on PROTAC degradation efficiency through phenotypic assay when a direct measurement is not possible.
- It provides an initial assessment of degradation efficiency based on limited affinity data, potentially minimizes unnecessary experimentation and accelerates the compound screening process.
- Future studies will focus to link this framework to a viral dynamic model to better anticipate the in vivo PD effect → guide dosing and sampling for animal experiments.

Acknowledgement

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