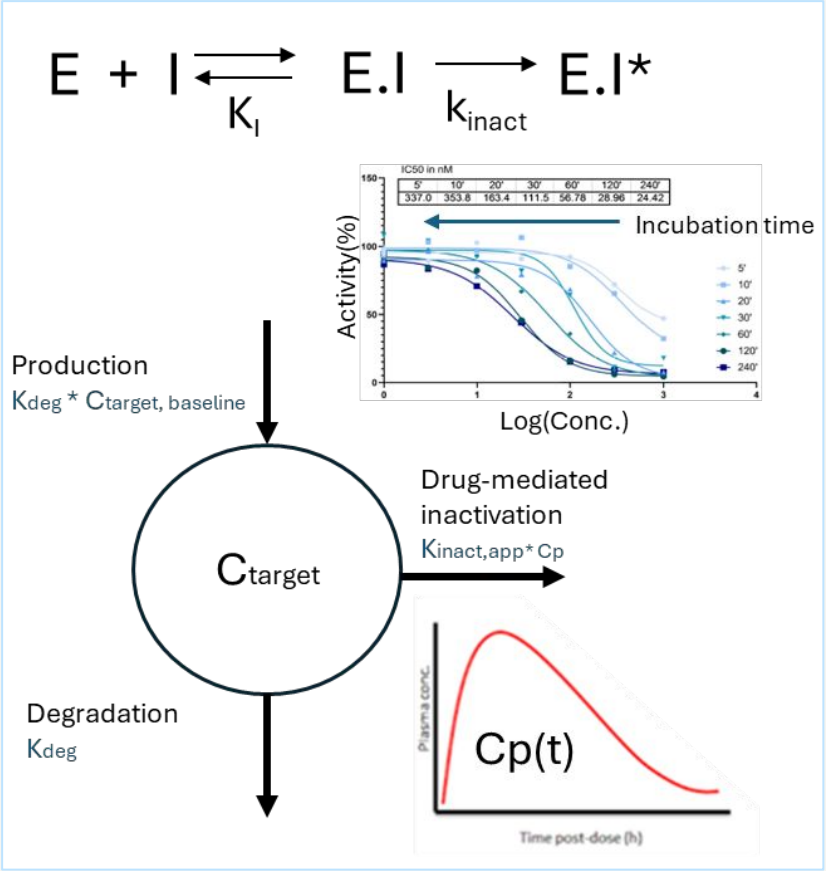


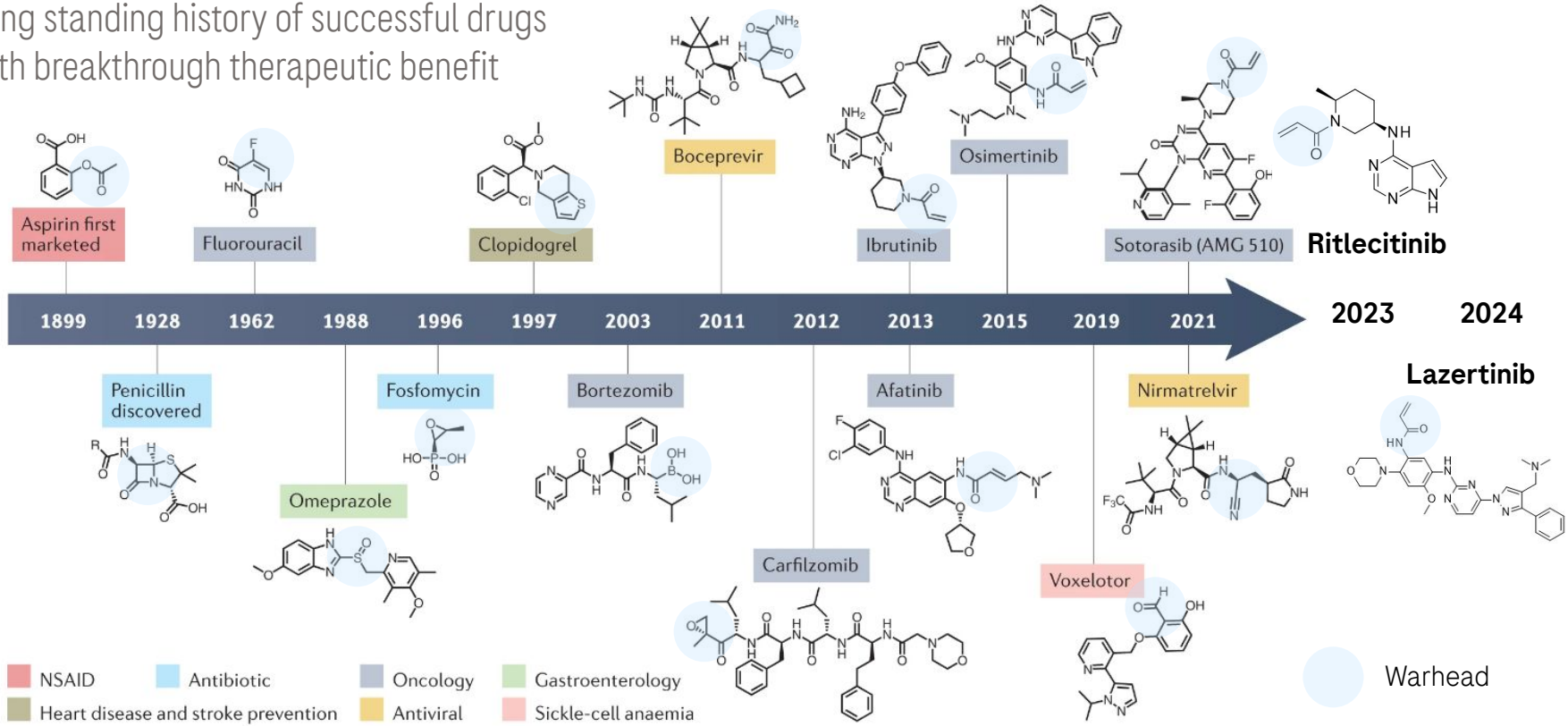
Defining Human Dosing for Covalent Inhibitors with Translational PKPD and Protein Turnover Data

Neil Parrott & Jitao David Zhang

April 2025 | DMDG Meeting *Focusing on Targeted Covalent Drugs*

Covalent Drugs on the Market

Long standing history of successful drugs with breakthrough therapeutic benefit



Nature Reviews Drug Discovery, 21, 881–898 (2022

Aims of this presentation

----------PART 1-NEIL----------

- Share how PK/PD modelling contributed to the selection and development of a covalent inhibitor
- Share how in vitro data, in vivo animal data and physiologically based PK and PD modelling were used to predict a human dose

----------PART 2-DAVID----------

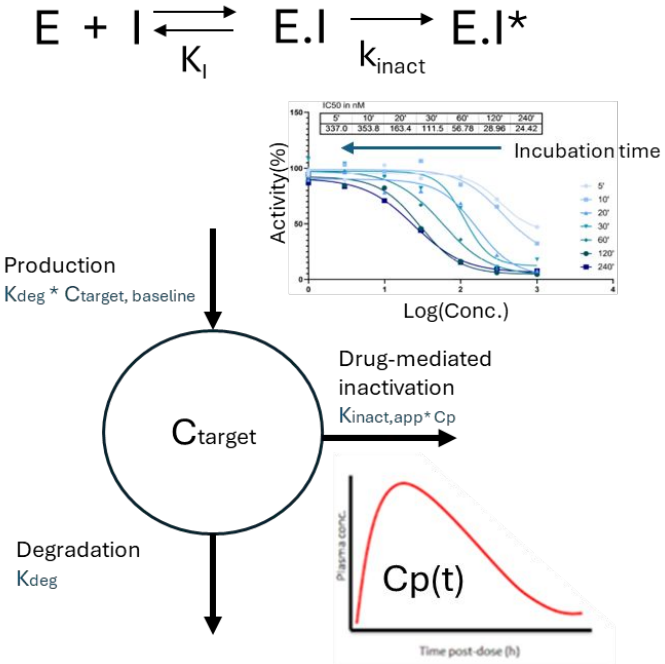
- Highlight the importance of target turnover
- Share efforts to build and apply target turnover data

How can PK PD modelling help ?

Questions to be addressed

- How do biochemical readouts of inhibition compare to cellular measures?
- How do in vitro measures translate to in vivo?
- How does target inhibition in vivo relate to efficacy?
- What is human PK and what dose will be needed?

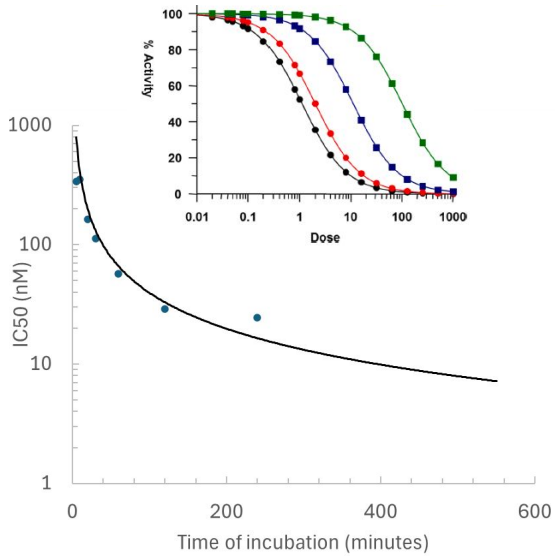
Complexities to be balanced



IC50 depends on incubation time

- IC50 is time dependent
- Time independent parameters are more complex to measure
  - Conc. for ½ maximal inactivation (Ki)
  - 1st-order inactivation rate constant (kinact)
- Measurement of kinact/KI require more resource
- However these parameters are related and correlated which can be useful for lead optimization

$$IC_{50(t)} = \frac{\ln(2) \times (1 + S/K_m)}{t \times k_{inact}/K_i}$$



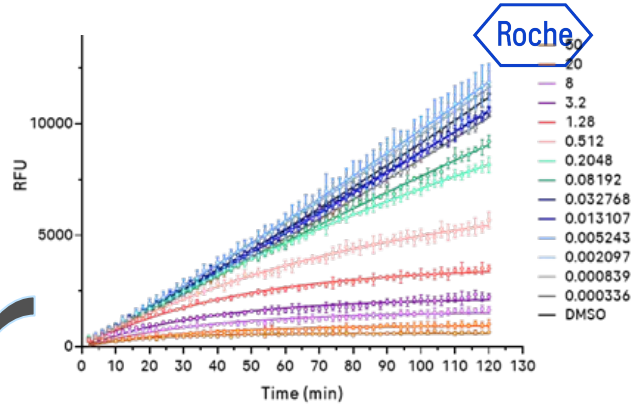
a plot of IC50(t) vs. 1/(kinact/Ki) is linear with slope affected by [S] and t.

A. Thorarensen et al., Bioorg. Med. Chem. 29 (2021) 115865.

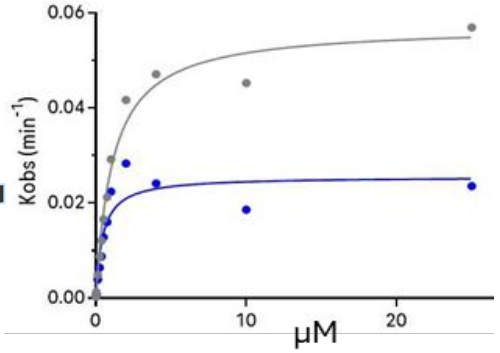
Human dose prediction - Roche Case Study

Early estimation of time dependent inhibition with biochemical assay

- Initial estimates of inactivation rate
- Enzyme activity measured using an ATP-dependent fluorescence-based assay employing recombinant enzyme and a fluorogenic substrate
- Used in initial simulations. Combining with estimated target half-life to explore PK requirements for sustained inhibition



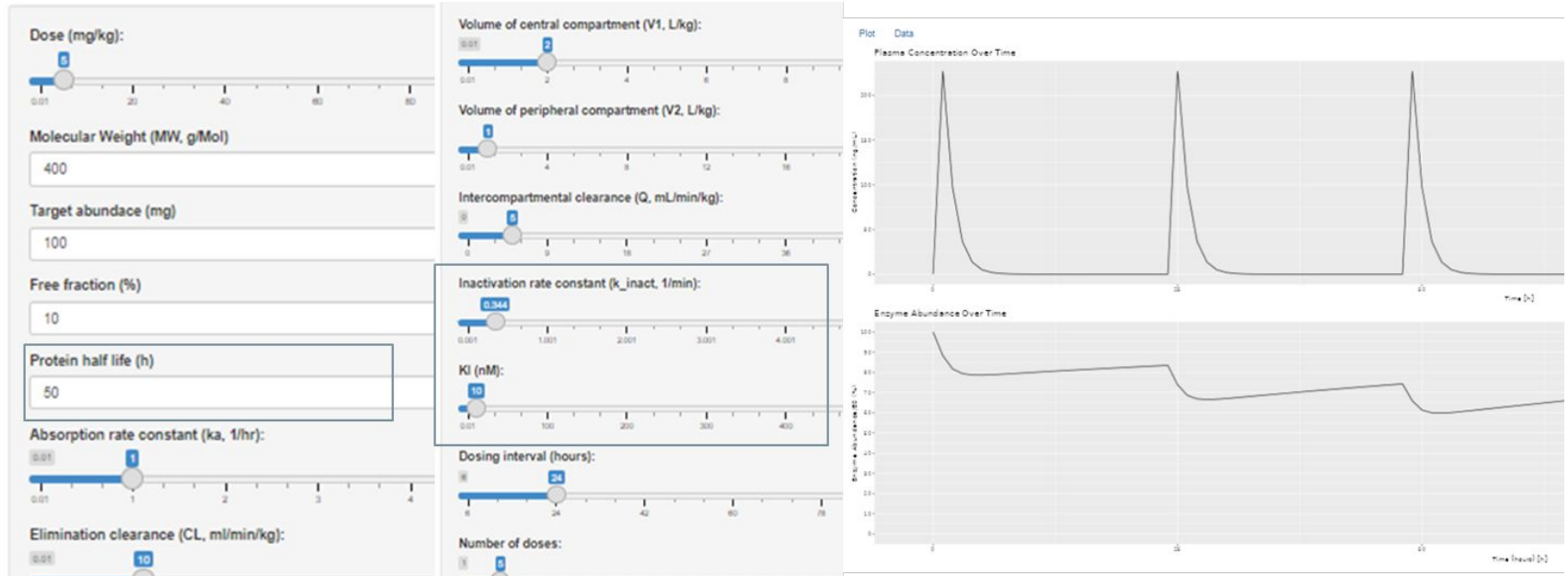
Kobs=observed rate constant



Kinact (s^{-1})	KI (M)
0.955×10^{-3}	1.108×10^{-6}

PK/PD modeling is needed to translate in vitro to to in vivo

Allows consideration of time dependent parameters (KI, kinact), together with target turnover estimates and in vivo pharmacokinetics

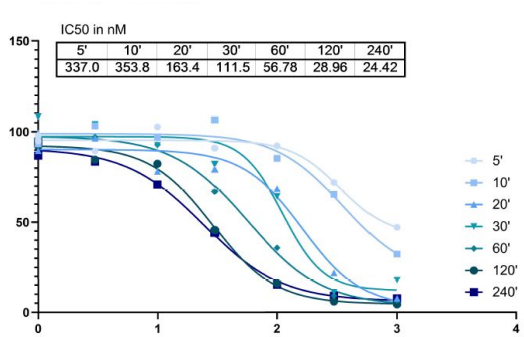


Shiny app. developed by Andres Olivares

Human dose prediction - Roche Case Study

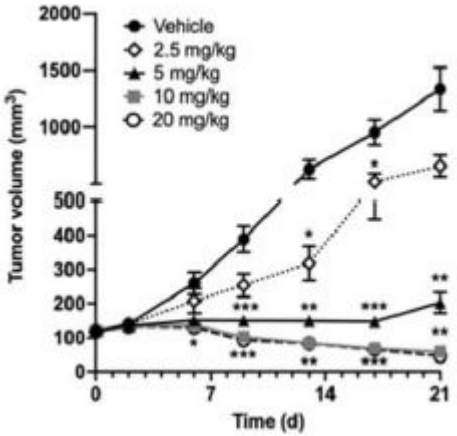
Refining inhibition parameters and linking to in vivo efficacy in mouse

cellular in vitro inhibition assay



Determination of inactivation parameters in the xenograft cell line

In vivo efficacy

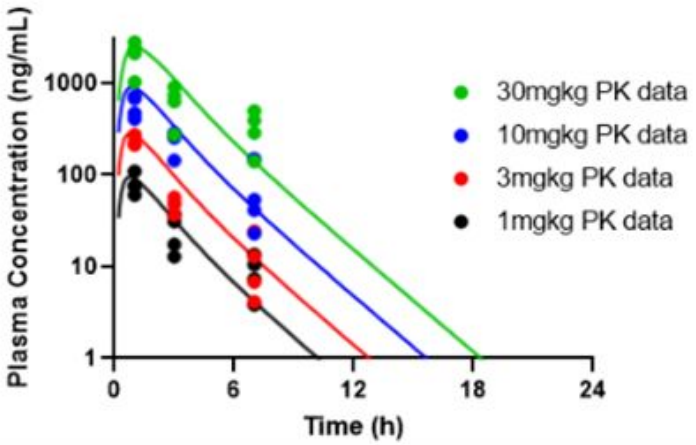


In tumor growth inhibition in xenograft mouse model

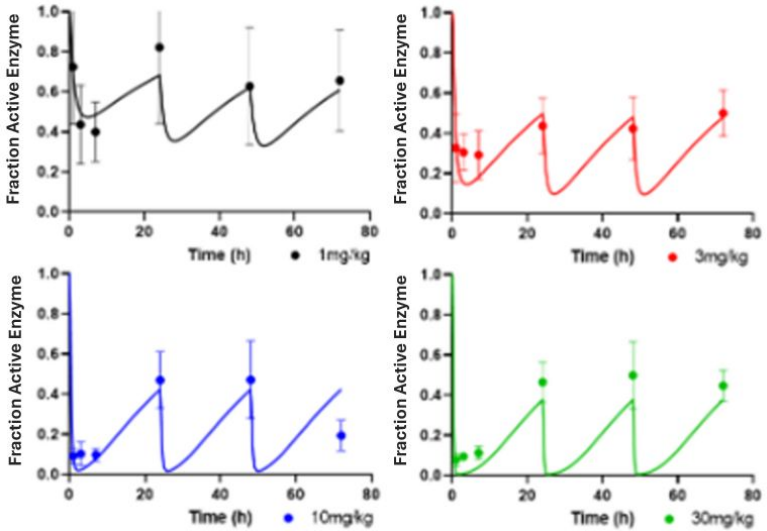
Human dose prediction - Roche Case Study

Confirming target inhibition in vivo

PKPD study in xenograft mouse

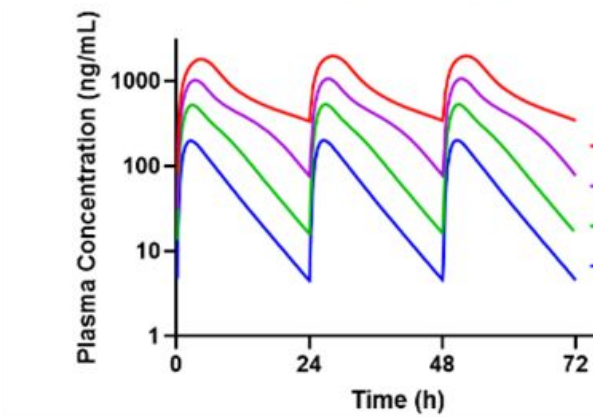


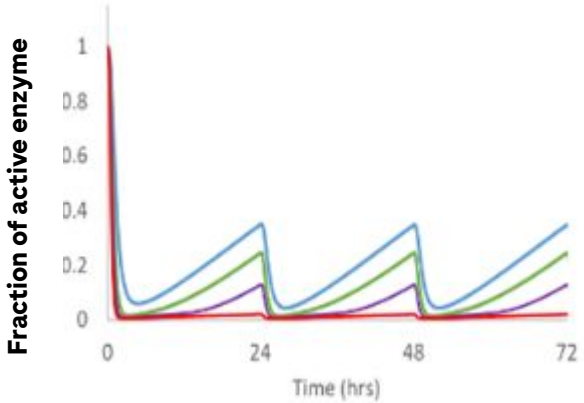
Verification of simulated target inhibition with measurements



Human PK and Target Inhibition Prediction - Roche Case Study

PBPK prediction of human PK and exploration of inhibition at different doses





Value for support of Phase 1 and Early Clinical Development

- Model parameter sensitivity analysis leveraged to explore the impact of uncertainties on dose and dosing regimens
  - Target engagement achieved with a different tumor penetration
  - q.d. vs b.i.d. dosing
- Rapidly update PK model with first clinical PK
  - Accounting for time and dose dependencies
  - PK and project to steady state with modelling
- Develop and verify more mechanistic QSP modeling approaches linking TE to tumor killing
- Transition from PBPK/PD to PopPK/PD considering variability

Summary of PK/PD modelling

- Important to understand target inhibition early to guide optimization
- Essential to take account of time dependent inhibition and target turnover
- Understand PK requirements and likely clinical dose range
- Simulation is needed to combine these complexities
- As project advances model input data is refined and model simulations verified vs cellular and in vivo data
- Simulations can guide dose estimation for clinical candidate and explore uncertainties
- Model refined with first clinical data and applied to further guide clinical development

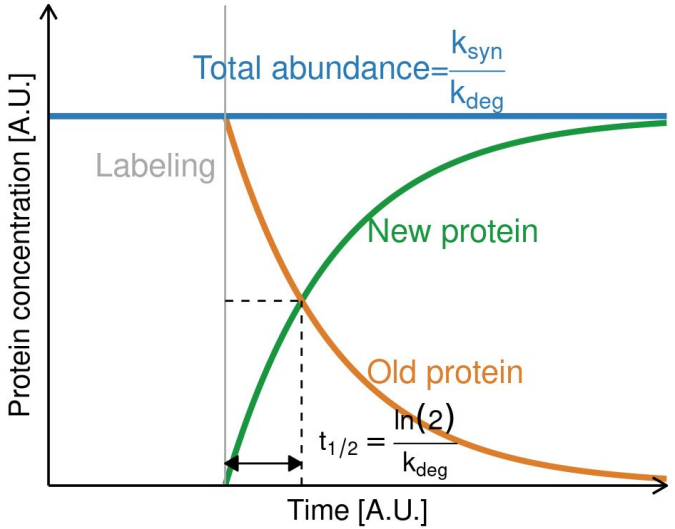
Acknowledgements to the pre-clinical and clinical modelling team & experimental experts:

Stephen Fowler, Matthias Wittwer, Christophe Meille, Gustavo Guerrero, Paul Grimsey, Mattia Berton, Matteo Berti,Piergiorgio Pettazzoni, Jasmin Emmenegger, Dominik Heer

Importance of protein turnover data

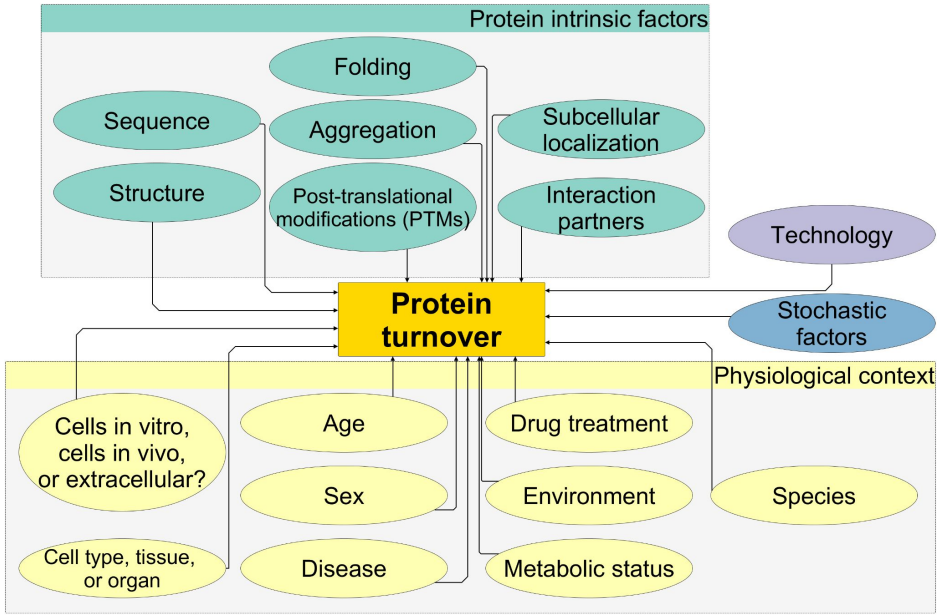
Protein turnover is critical for drug discovery & development

- Protein turnover affects efficacy, potency, ADME properties, and safety profiles of drug candidates.
- Protein turnover is essential for target prioritization and modality selection, for instance covalent binders and/or targeted protein degraders.
- Understanding protein turnover helps to translate pharmacokinetic and pharmacodynamic (PK/PD) relationships between systems.



Assumptions: zero-order synthesis (rate k_{syn}), first-order degradation (rate k_{deg}), and steady state (i.e. no expression changes).

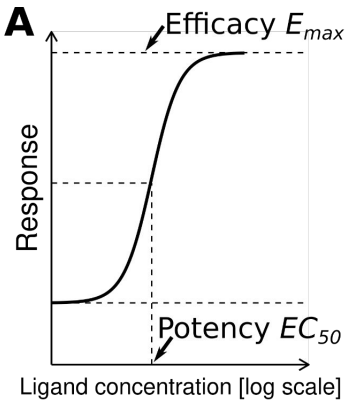
Half-life varies between proteins and contexts: influencing factors and an example

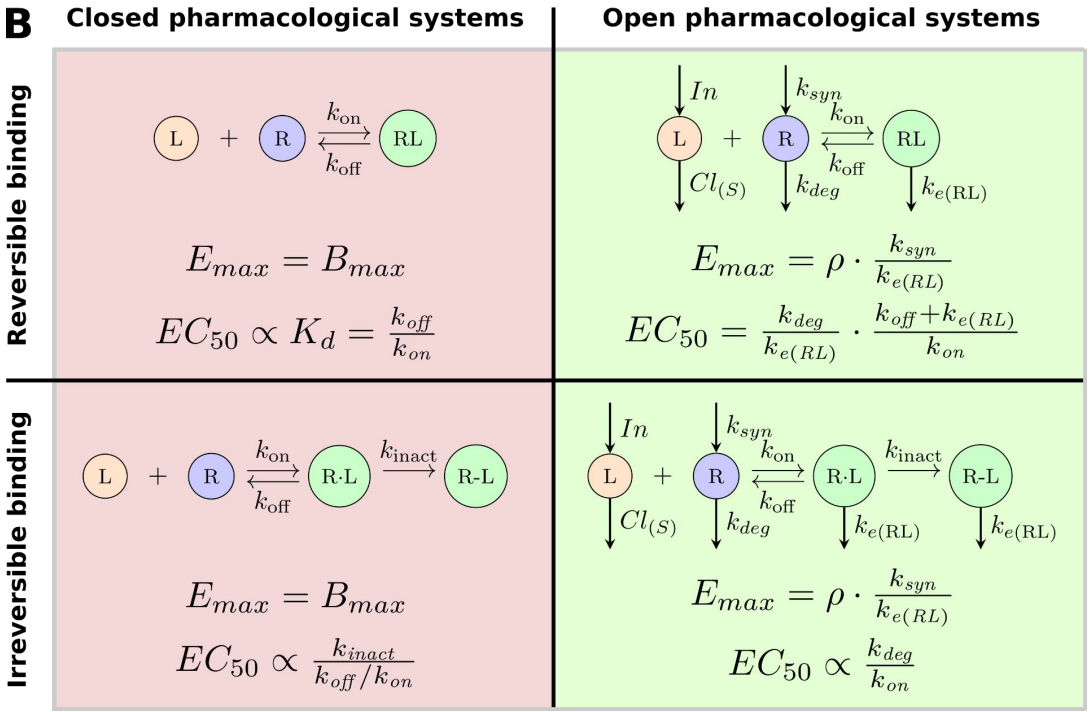


Condition	Half-life of protein X	Source
Human neurons *in vitro*	38.6h	Roche in-house data, courtesy of Lothar Lindemann and Sarah Morillo Leonardo
Mouse neurons in vitro	34.1h (standard error:3.9h)	Fornasiero et al., Nature Communications, 2018
Mouse cortex *in vivo*	619.2h (25.8d)	Kluever *et al.*, Science Advances, 2022

Open models integrate protein turnover into pharmacological modeling

According to open models (see the comprehensive review by Gabrielsson and Hjorth), target turnover impacts *in vivo* potency, efficacy, and clearance.





Predictions by open models

Highlighted in blue: particularly relevant for covalent binders

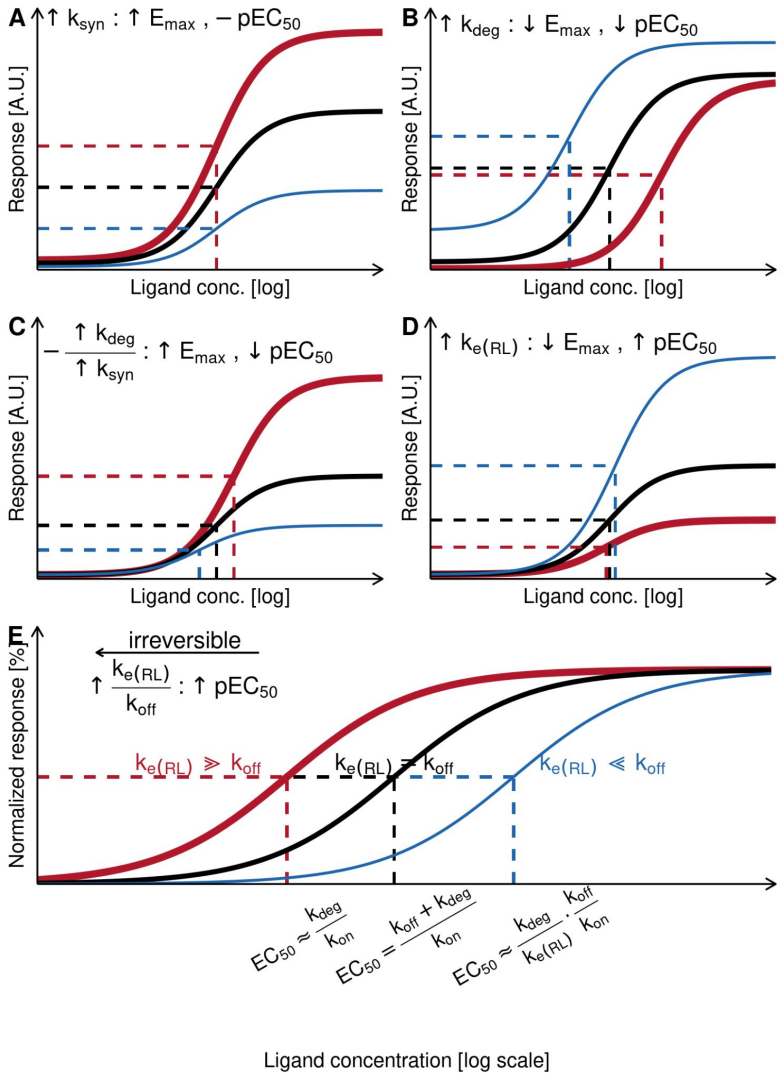
A. Higher target synthesis rate increases efficacy while potency remains unchanged.

B. Higher degradation rate decreases both efficacy and potency.

C. Keeping the steady-state abundance fixed, increasing both synthesis & degradation rates increase both efficacy and potency.

D. Higher ligand-target complex elimination rate reduces efficacy while increases potency.

E. Potency of covalent inhibitors is dictated by k_{deg}/k_{on}: slow turnover and fast on-rate are preferred.



Roche's Protein Turnover Database integrates external and internal data

The table shows the protein half-life datasets that David curated for the turnover database. The curation contains following steps:

1. The data were curated from individual studies.
2. Features (uniprot IDs, protein groups, etc.) were harmonized and mapped to genes of the respective genome as well as to human orthologues.
3. Units of measurements were harmonized to hours.
4. Sample annotations are harmonized.

Roche Protein Turnover Database

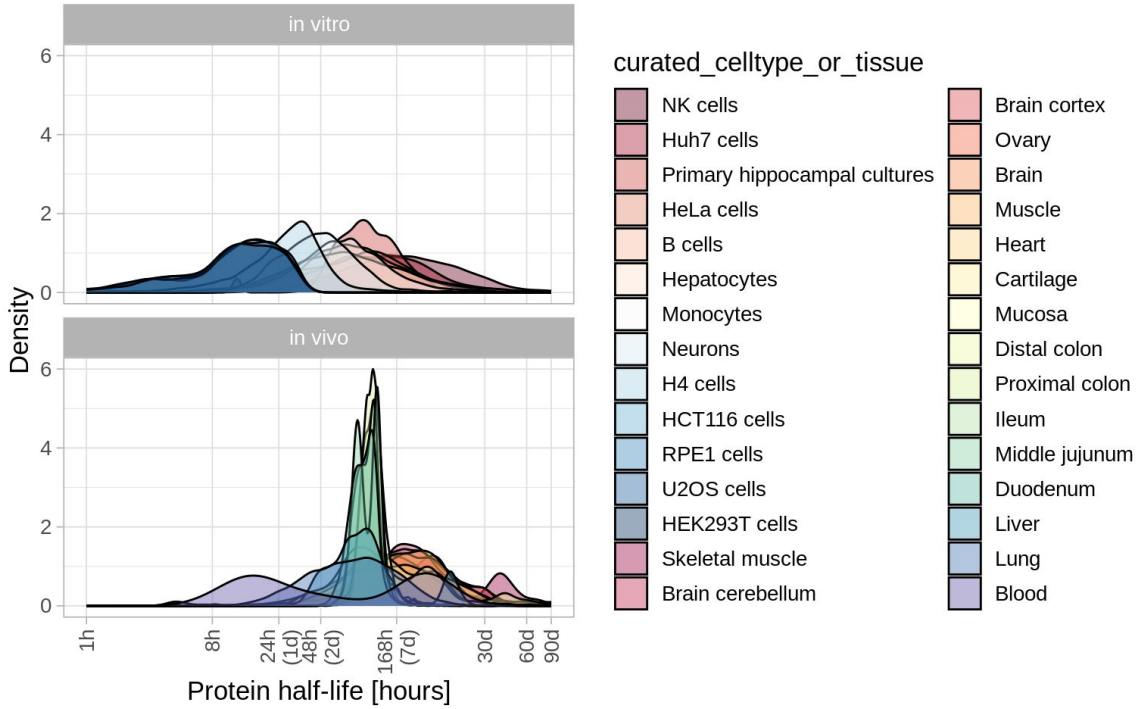
Dataset overview (v202407)

	organism	assay_type	celltype_or_tissue
Doerrbaum-2018	rat	in vitro	Primary hippocampal cultures
Fornasiero-2018	mouse	in vivo	Brain cortex, Brain cerebellum, Heart, Muscle
Mathieson-2018-human	human	in vitro	NK cells, Hepatocytes, Monocytes, B cells
Mathieson-2018-mouse	mouse	in vitro	Neurons
Arike-2020	mouse	in vivo	Duodenum, Middle jujunum, Ileum, Proximal colon, Distal colon
Li-2021	human	in vitro	U2OS cells, HEK293T cells, HCT116 cells, RPE1 cells
Morgenstern-2021	human	in vitro	HeLa cells, Huh7 cells
Rolfs-2021	mouse	in vivo	Cartilage, Skeletal muscle, Mucosa, Liver, Blood
Kluever-2022	mouse	in vivo	Brain cortex, Brain cerebellum
Chen-2023	mouse	in vivo	Lung, Heart, Brain
Harasimov-2024	mouse	in vivo	Ovary
Lothar-H4	human	in vitro	H4 cells

We observe in general longer half-life *in vivo* than *in vitro*, with variations between cell/tissue types

Right: density plot of protein half-life, stratified by assay type (*in vitro* versus *in vivo*) and by cell type or tissue.

Most *in vivo* studies tend to report longer half-life than at least some *in vitro* studies, though considerable variability is observed in both categories.



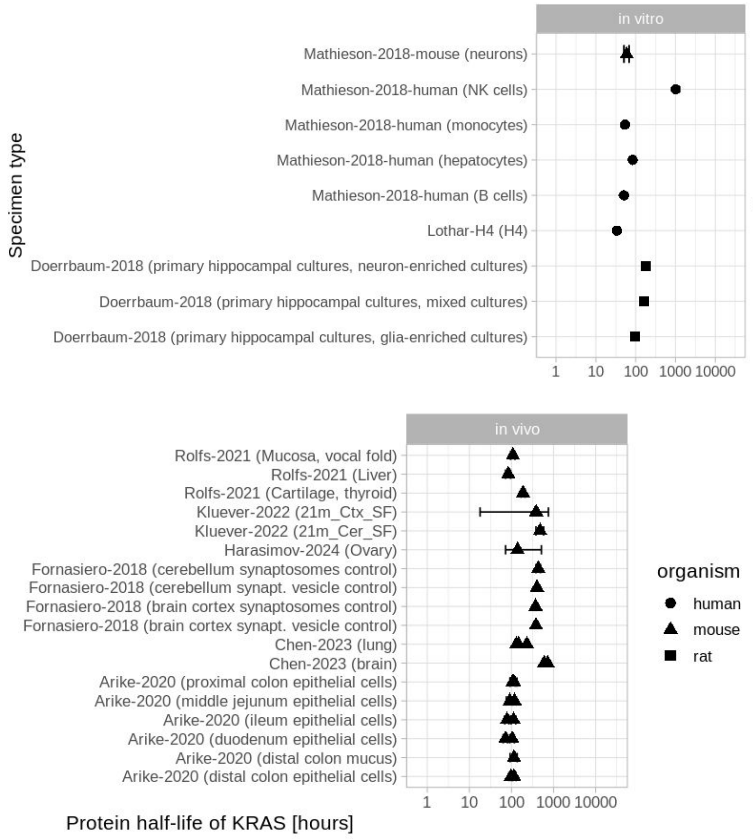
A survey of half-life of covalent binder targets

We curated 31 covalent binders which are either in clinical development or approved, targeting a total of 26 human and 7 viral or bacterial proteins.

The table summarizes half-life data for 24 human proteins. Turnover data of KRAS is visualized with boxplots.

Median half-life of targets of covalent binders [hours]

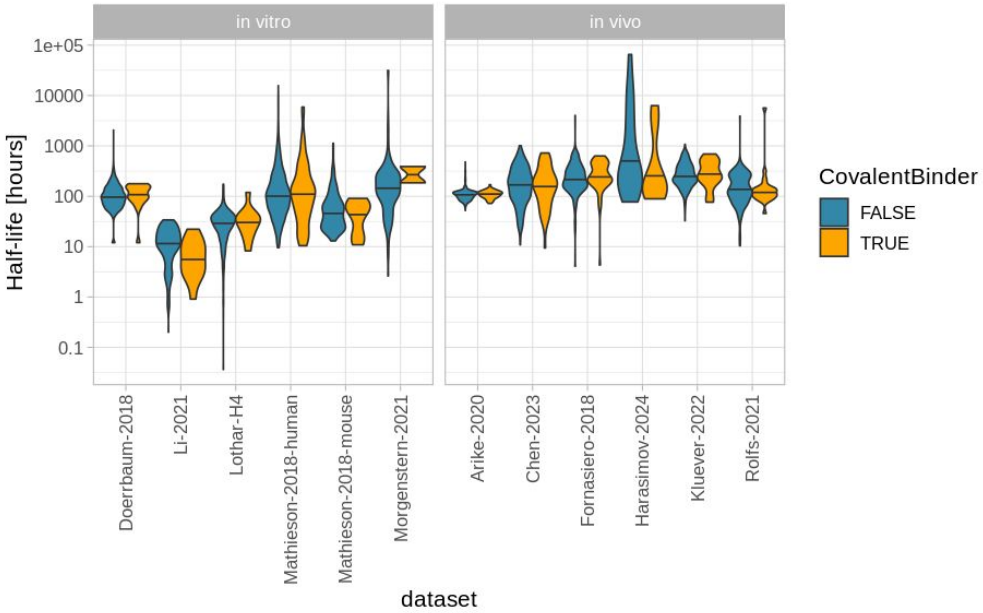
	unique_drugs	in vitro	in vivo
EGFR	8	35.0	64.9
ERBB2	4	17.8	NA
BTK	3	79.2	NA
KRAS	3	84.3	124.6
PSMB5	3	109.7	212.6
ERBB4	2	19.3	70.2
MAOB	2	111.8	272.6
P2RY12	2	194.8	167.3
PSMB1	2	129.9	163.9
ABAT	1	185.0	433.6
ATP4A	1	NA	136.8
FGFR4	1	7.7	NA
HBA1	1	119.2	NA
HMGCR	1	10.6	5648.8
JAK1	1	12.5	89.1
JAK2	1	57.0	NA
JAK3	1	10.4	NA
PSMB10	1	160.7	46.9
PSMB2	1	133.3	197.5
PSMB8	1	119.8	128.5
PSMB9	1	203.2	266.1
PTGS1	1	667.6	145.1
PTGS2	1	8.2	NA
TYK2	1	20.5	NA



Targets of covalent binders have comparable half-life with targets of non-covalent binders, yet short-living proteins are less targeted by the covalent approach

The violin plot compares the half-life of targets of covalent binders (N=24) with the half-life of targets of non-covalent molecules for which a high potency or functional inhibition (pACT>=8, N=788).

Targets of covalent binders and those of non-covalent drugs have in general comparable half-lifes. However, covalent drug targets are devoid of shortest-living proteins.

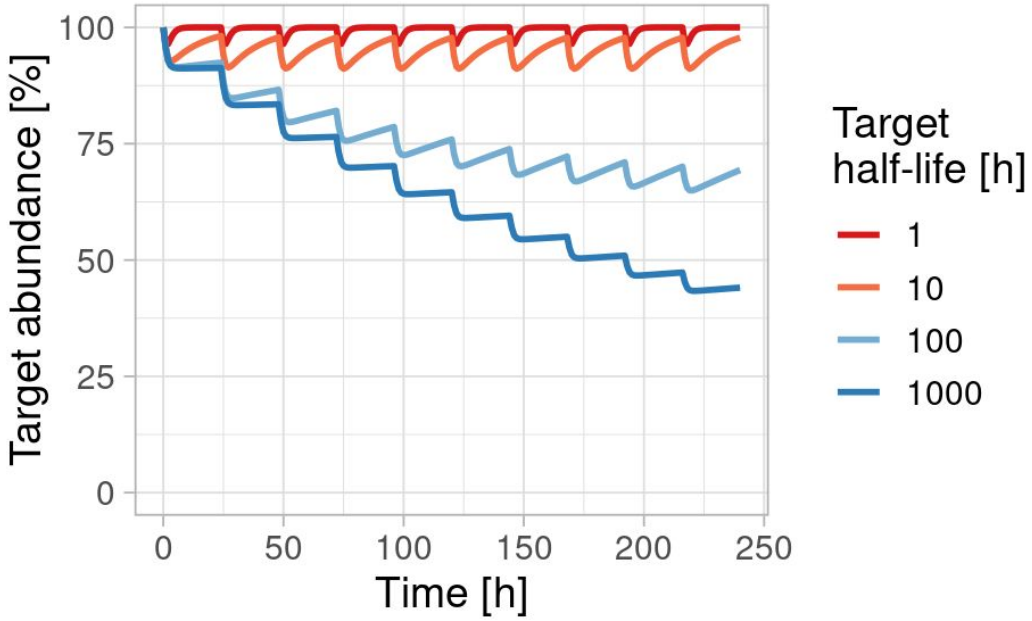


Protein half-life can be integrated into PK/PD models

Example: target degradation PK/PD model of covalent binding by Andrés Olivares (mentioned before by Neil)

Modelling and simulation suggests that the PD effect of target degradation by covalent binder is sensitive to the protein half-life.

Publication from Bayer colleagues, kindly shared by Miro Eigenmann, also suggested that protein half-life is a key parameter that affected the predictions of their mechanistic PD model for targeted protein degraders.



Summary & Conclusions

1. PK/PD modelling contributed to the selection and development of a covalent inhibitor.
2. *In vitro* data, *in vivo* animal data and physiologically based PK and PD modelling were used to predict a human dose.
3. Target protein turnover affects potency and efficacy of drugs.
4. Protein turnover varies among proteins and by physiological contexts.
5. Integrating parameters of protein turnover into PK/PD modelling bears the potential to empower covalent drug discovery.

Doing now what patients need next