

Introduction to DMPK Practices for Proteolysis Targeting Chimera Molecules

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WuXi AppTec DMPK



OUTLINE

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PROTAC and Development History

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WuXi AppTec DMPK Facilities and Capabilities

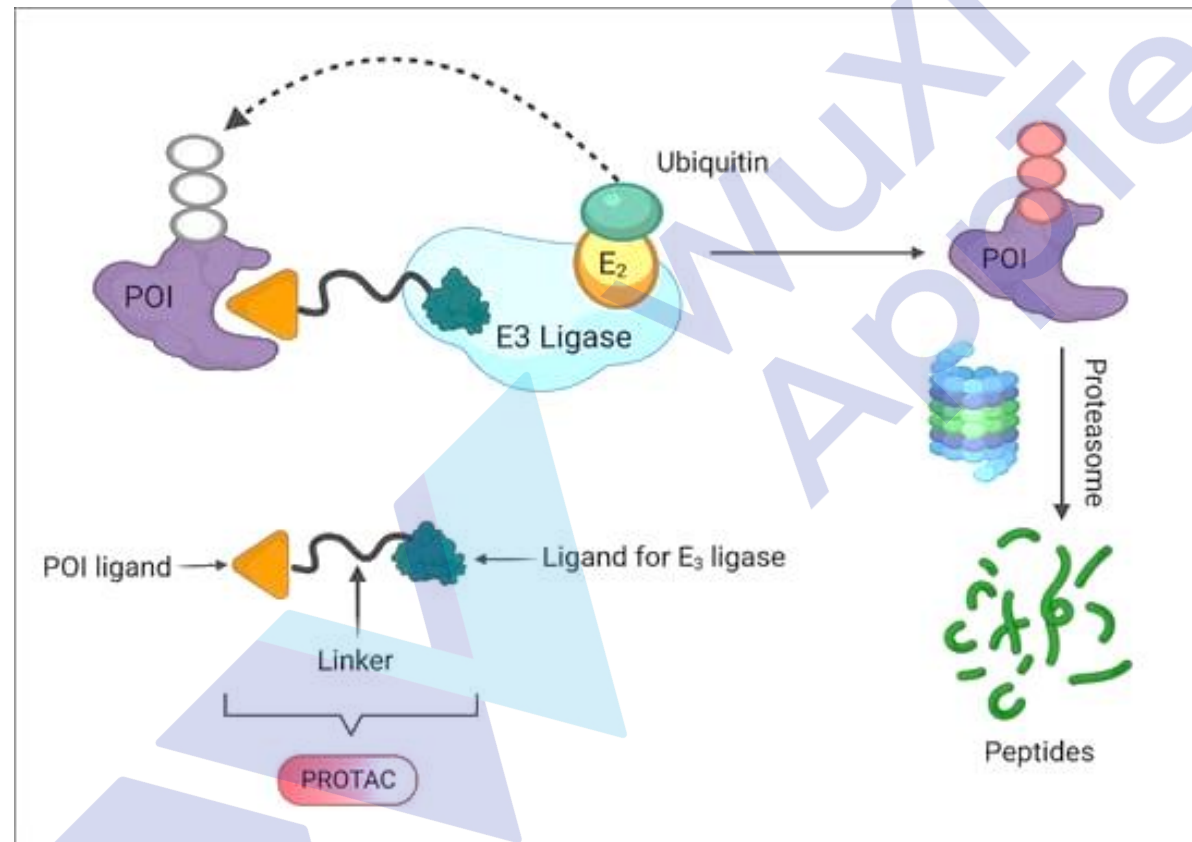
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ADME-PK Solutions for PROTACs

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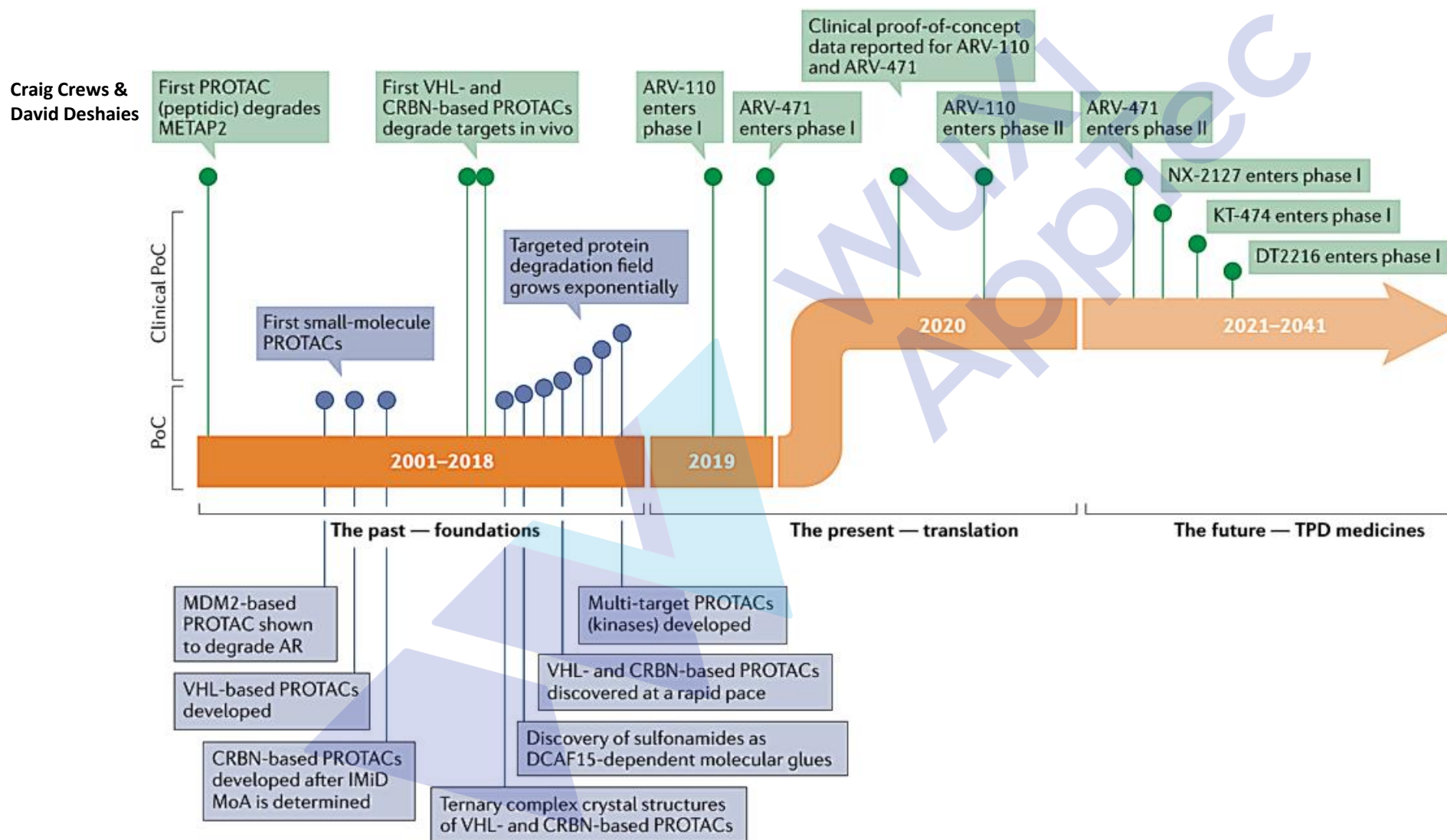
Summary of DMPK Study Strategy for PROTACs

PROTACs (Proteolysis Targeting Chimeras)



Sincere, NI, et al. *Molecules* **2023**, *28*, 4014

History of PROTAC Molecule Development



Békés M, et al. *Nat Rev Drug Discov.* 2022 Mar;21(3)

WuXi AppTec DMPK Facilities and Capabilities



200+

Assay types



300k+

In vitro studies each
year



20k+

In vivo studies each
year



180k+

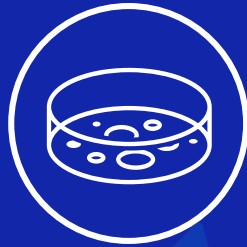
Samples analyzed per
week

WuXi AppTec DMPK Figures in PROTACs



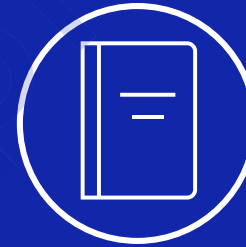
10+

Years experience in bio-analysis



2000+

PROTAC molecules each year



27,000+

Screening molecules by Dec 2025



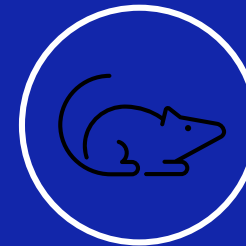
30+

IND filing molecules by June 2025



60+

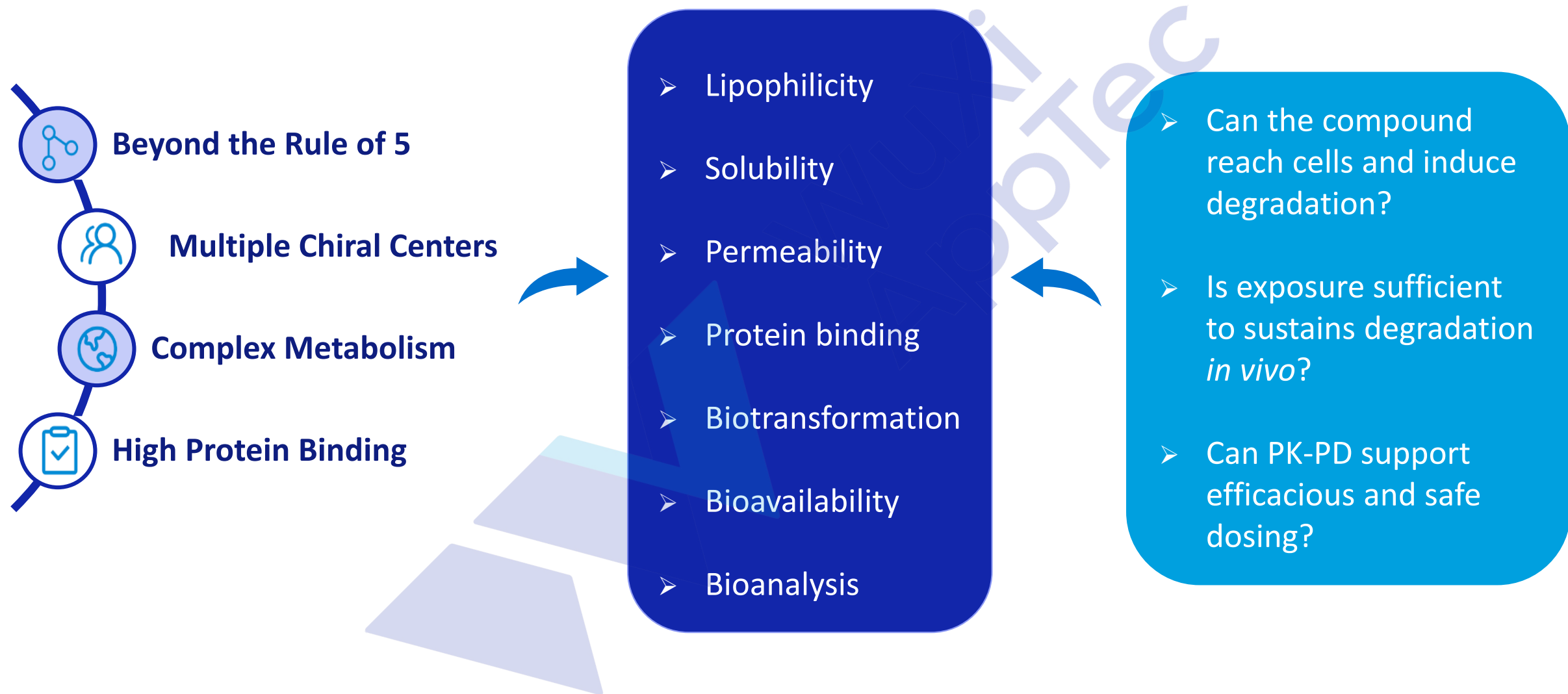
Global clients of PROTACs



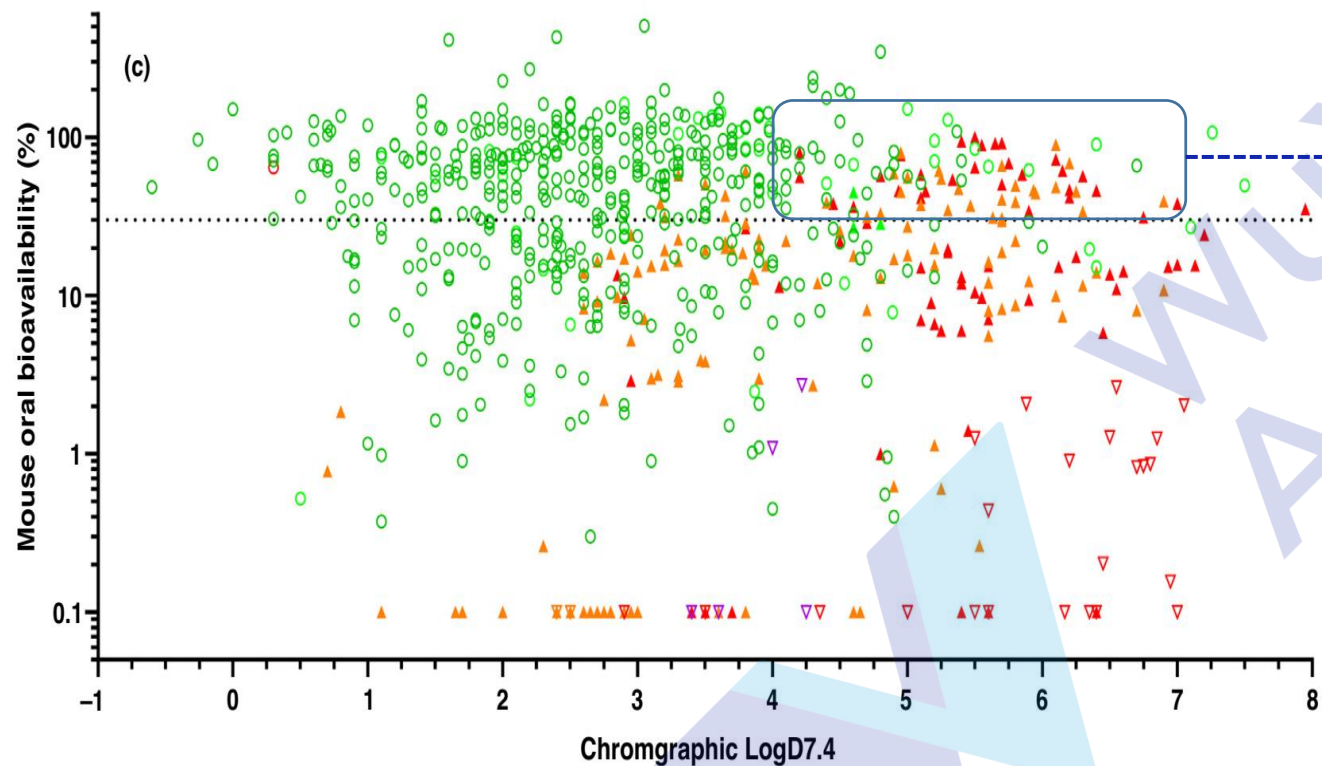
3-7

Working days for *in vitro* assays and *in vivo* PK studies

Unique DMPK challenges for PROTACs



ADME-PK Solutions for PROTACs _ Lipophilicity



Drug Discov Today. 2020 Oct; 25(10): 1793-1800.

Small molecules (open circle)
CRBN PROTACs (solid triangle)
VHL PROTACs (inverted open triangle)

- LogD 7.4 of most PROTACs are ranged from 1 to 7
- PROTACs with high lipophilicity achieved high bioavailability

ADME-PK Solutions for PROTACs _ Lipophilicity

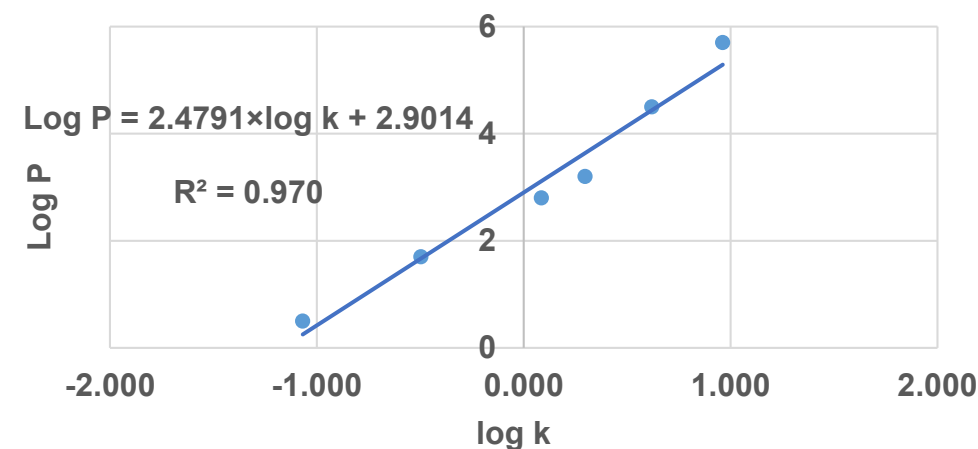
Methods	Estimated Range (log units)	Distraction	Speed	Sample Amount	Reproducibility	Predictability
<i>In silico</i> prediction	wide	--	rapid	--	--	★
Shake flask	-2-4	not suitable for degradable compounds	time consuming	small	★★	★★★★
Reverse-phase HPLC	0-7	friendly; insensitivity to impurities	rapid	small	★★★	★★

RP-HPLC method for Log P determination

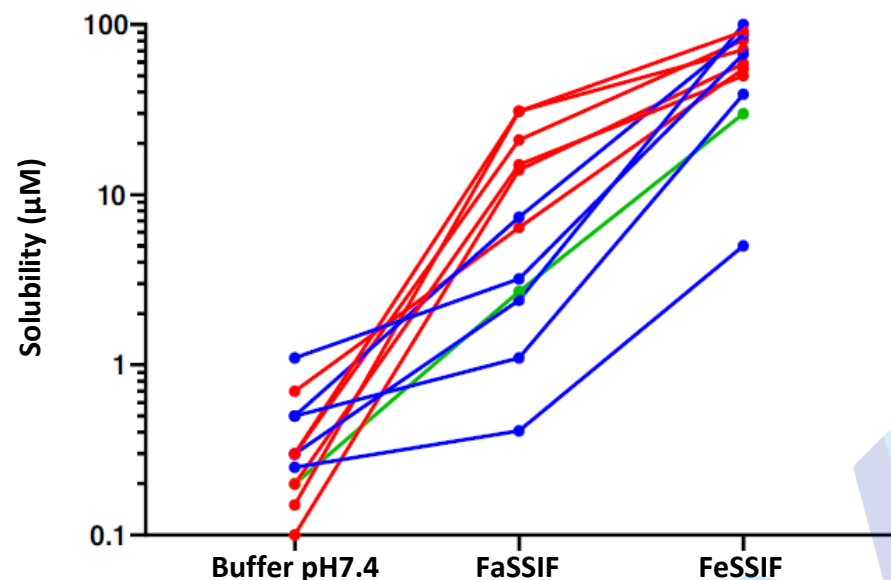
$$\log k = \log(t_R - t_0) / t_0 \quad (1)$$

$$\text{Log } P = a * \log k + b \quad (2)$$

A good linearity ($R^2 > 0.9$)



ADME-PK Solutions for PROTACs _ Solubility



Drug Discov Today. 2020 Oct; 25(10): 1793-1800.

- PROTACs had improved solubility in bio-fluids, especially in Fed State Simulated Intestinal Fluid (FeSSIF).

Solubility Evaluation

■ Method

- 24h Shake-Flask Method

■ Medium

- Aqueous Buffers (pH1-11)
- SGF, Simulated Gastric Fluid (pH1.2)
- SIF, Simulated Intestinal Fluid (pH6.8)
- FaSSIF, Fasted State Simulated Intestinal Fluid (pH6.5)
- FeSSIF, Fed State Simulated Intestinal Fluid (pH5.0)

ADME-PK Solutions for PROTACs _ Permeability

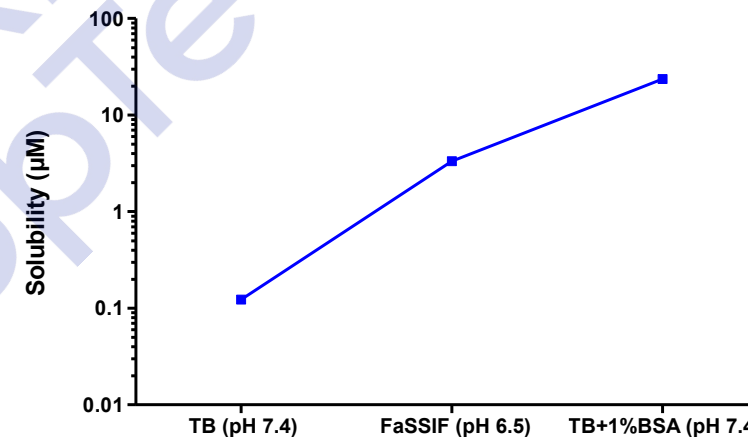
■ Challenges in Caco-2 Permeability Assays

- Low aqueous solubility at pH7.4
- High non-specific binding (NSB)
- Low recovery

■ Methods

- The addition of 1% BSA can improve solubility of PROTACs and reduce NSB.
- The use of FaSSIF (pH6.5) as donor solution and transport buffer (TB) with 1% BSA as receiver solution can improve solubility and reduce NSB.
- The addition of a pre-incubation of 20-24 hours would help the unbound intracellular concentration of PROTACs with LogP values >5 to reach a steady state, resulting in more reliable results.

Solubility of AVR-110



Compound ID	Buffer	%Binding
ARV-110	Transport Buffer (pH 7.4)	48.9
	Transport Buffer with 1% BSA	12.8

WuXi AppTec DMPK in-house data

ADME-PK Solutions for PROTACs _ Permeability

Compound ID	Caco-2 Permeability	Mean P_{app} (10^{-6} cm/s)		Efflux Ratio	Mean %Solution Recovery	
		A to B	B to A		A to B	B to A
ARV-110	TB (pH7.4) (Routine Method)	0.00864	0.333	38.6	25.3	20.6
	TB with 1%BSA in both Chambers (Solution 1)	0.00991	0.811	81.8	66.3	75.3
	FaSSIF in A Chamber, and TB with 1%BSA in B Chamber (Solution 2)	0.00328	/	/	52.1	/
	20-24 hr pre-incubation, TB with 0.25%(w/v) BSA in both Chambers (Solution 3)	0.0201	5.86	291	73.6	74.5

WuXi AppTec DMPK in-house data

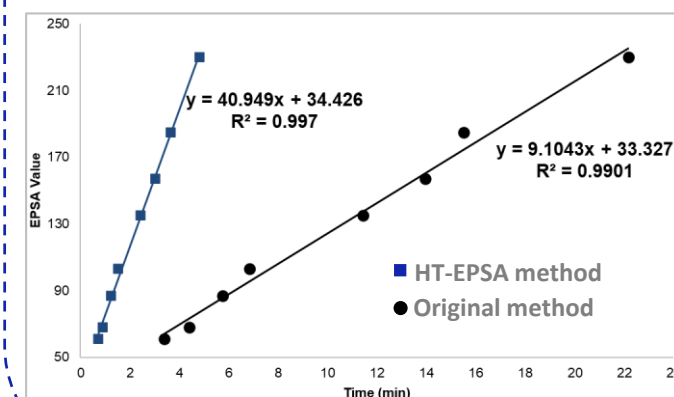
- Solution 1 is mainly applied for PROTACs, and solution 3 can be used as an alternative, in case PROTACs have higher Log P values, especially >5.
- Experimental polar surface area (EPSA) can also be used as a surrogate for intestinal permeability.

EPSA

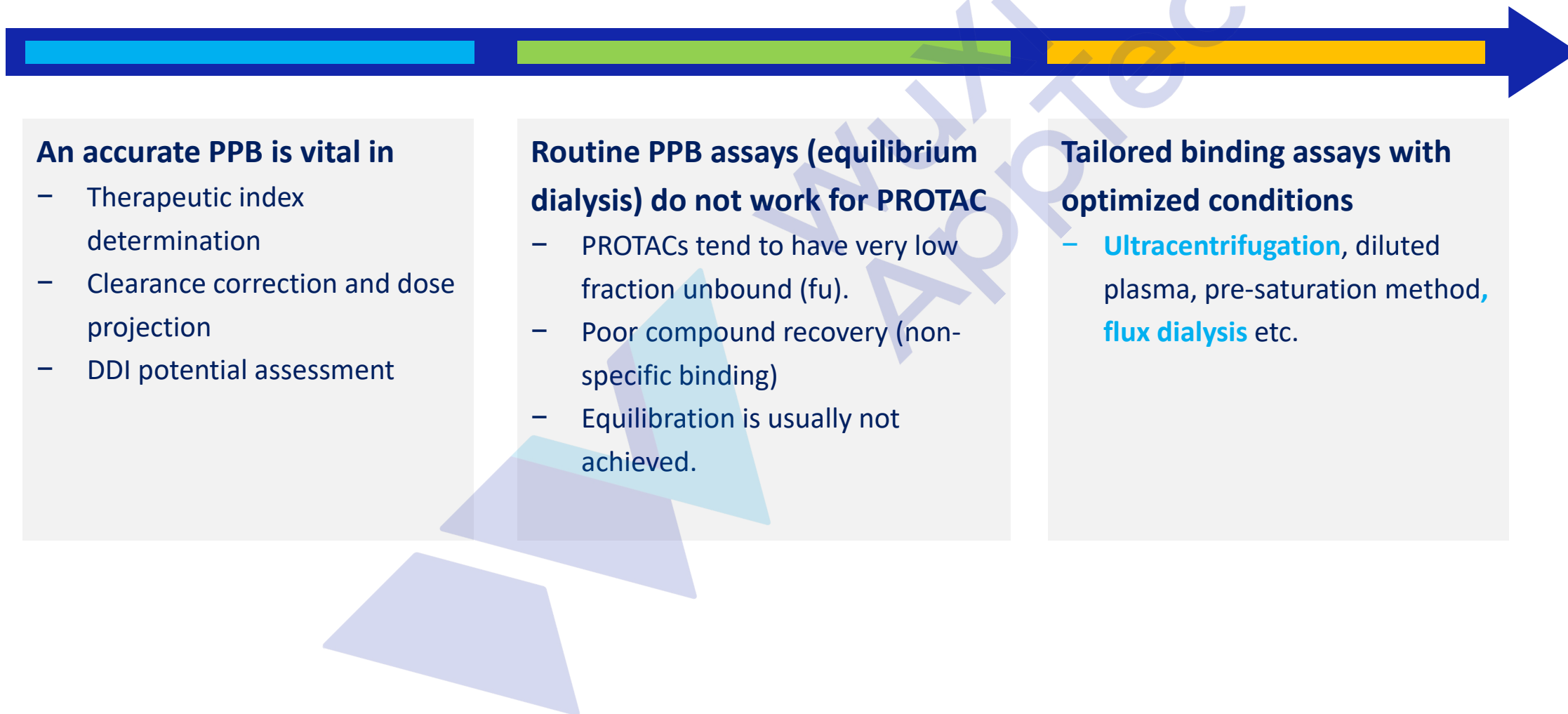


• UPCC-PDA

• UPCC-MS/MS

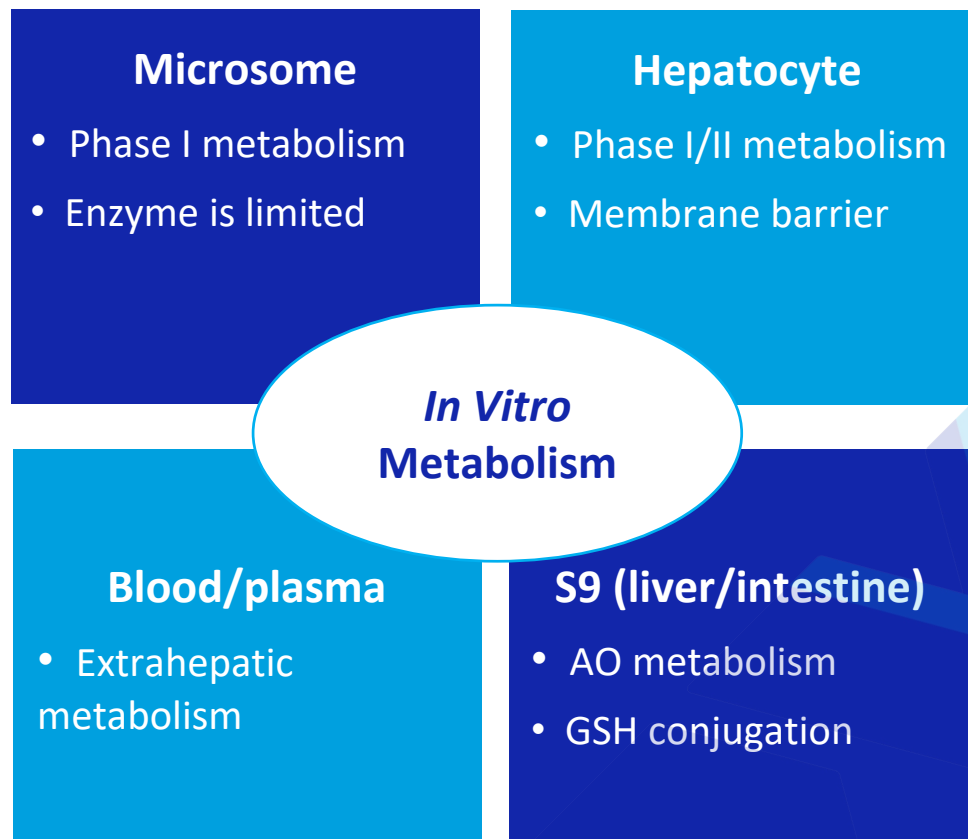


ADME-PK Solutions for PROTACs _ Protein Binding



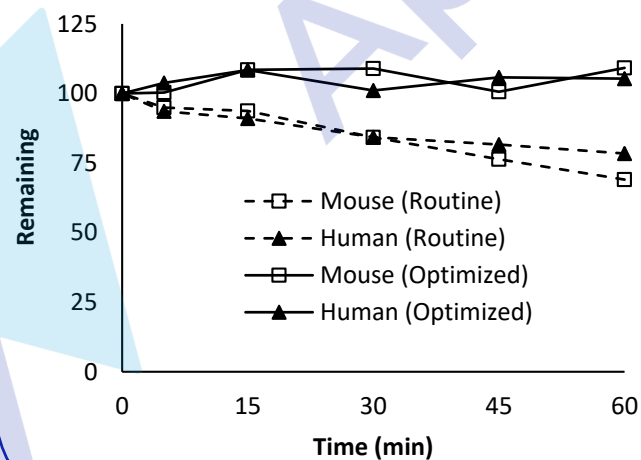
J Pharm Sci 104(8): 2627-36 (2015)

ADME-PK Solutions for PROTACs _ Biotransformation

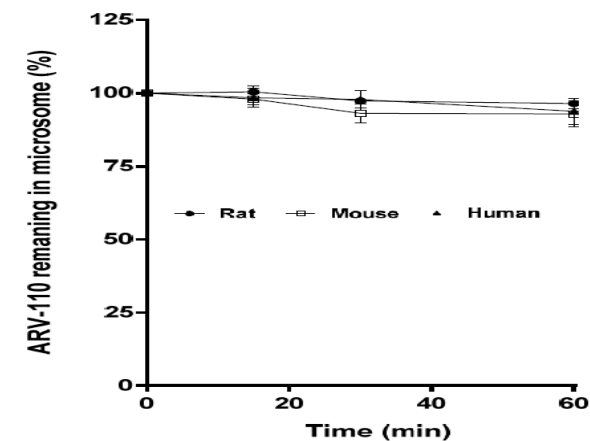


■ Plasma Stability of ARV-110

• WuXi AppTec DMPK in-house data

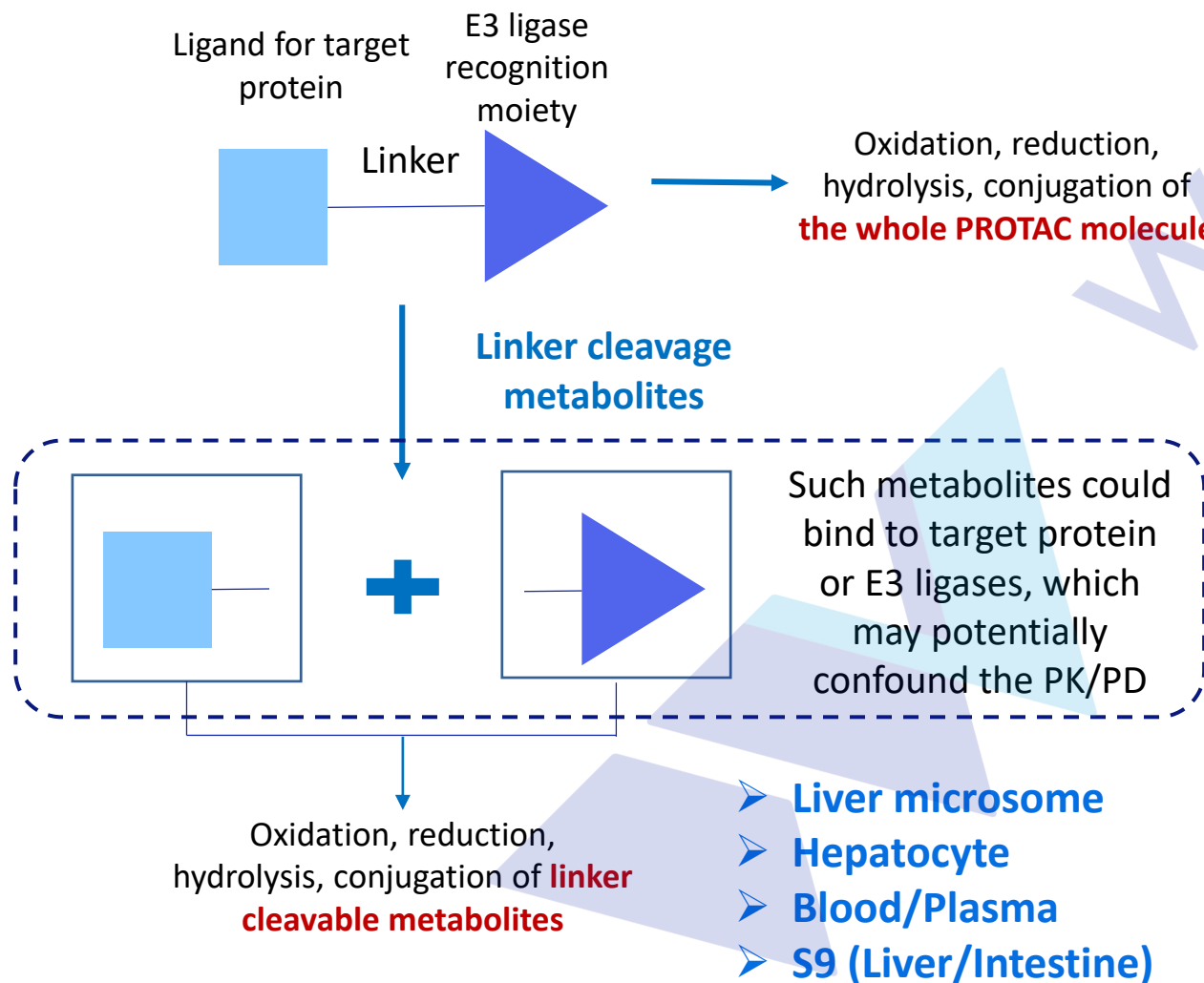


• Literature data



Molecules, 2022, 27(6): 1977.

ADME-PK Solutions for PROTACs _ Biotransformation



Soft spot identification in early structural optimization

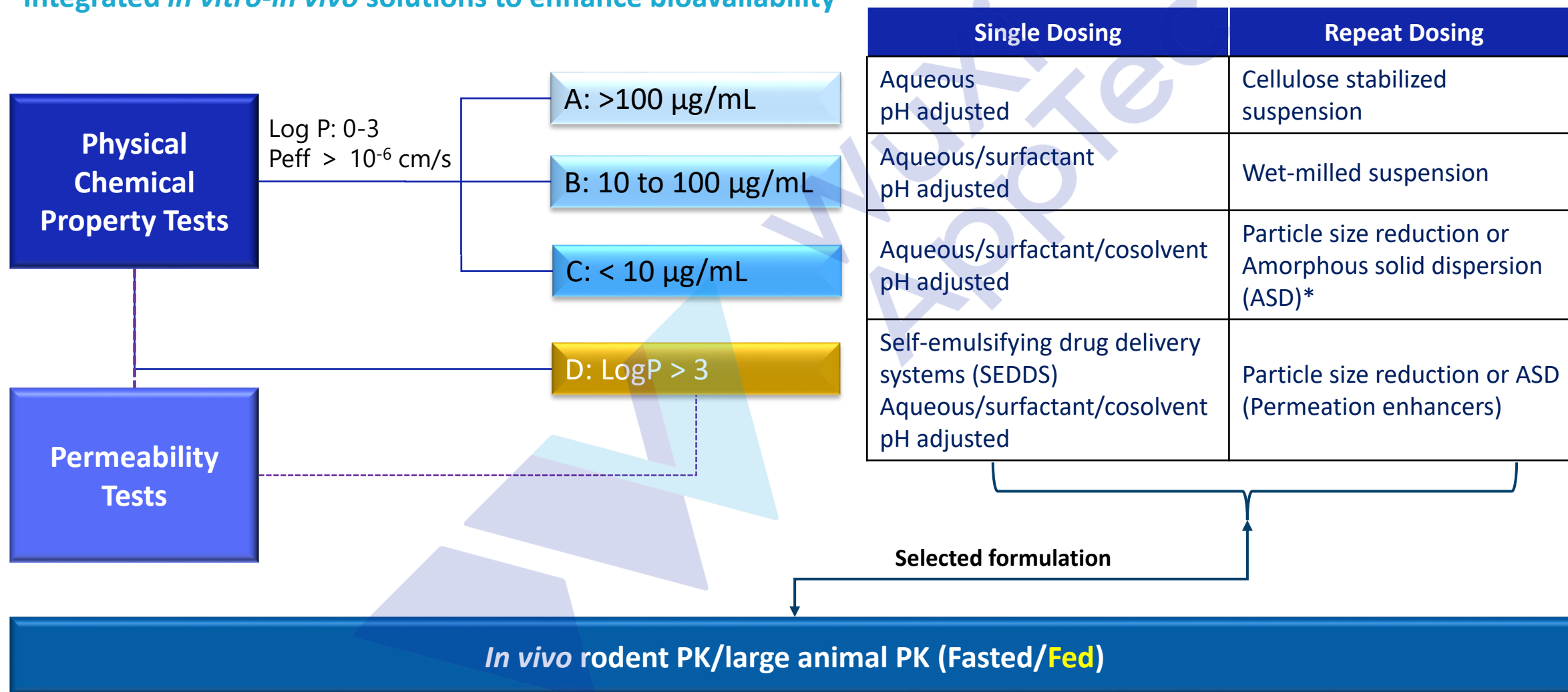
- Find ≤ 5 metabolites
- Find the soft spot for structure optimization
- Rapid and low cost

Comparison across species

- Metabolite profiling and structure elucidation of the full panel of metabolites
- Tox species selection
- *In vitro-in vivo* correlation in metabolism

ADME-PK Solutions for PROTACs _ Bioavailability

Integrated *in vitro-in vivo* solutions to enhance bioavailability



* Work with STA on ASD formulation development

ADME-PK Solutions for PROTACs _ Bioavailability

Solution

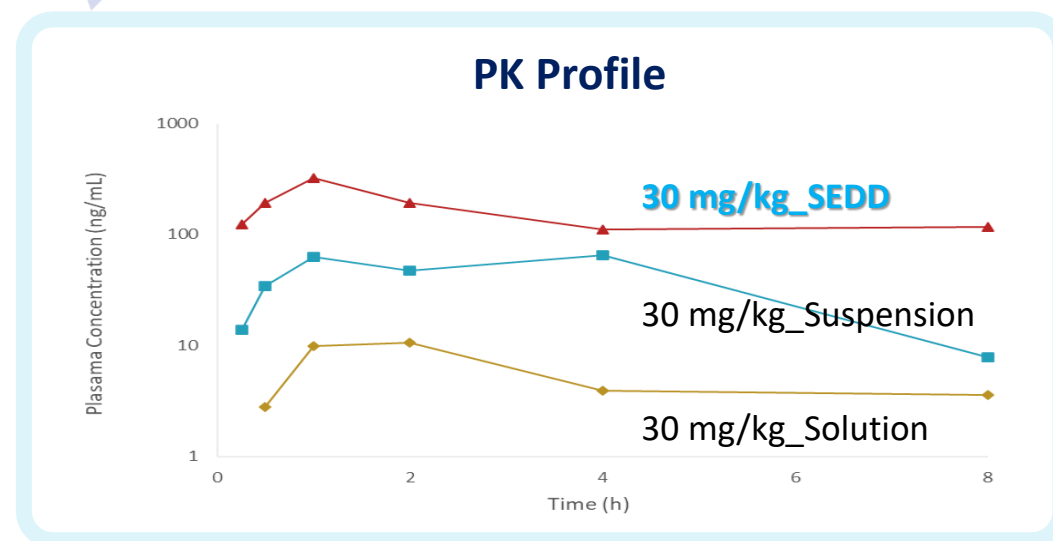
SEDDS

Suspension

Formulation	Dose (mg/kg)	C _{max} (ng/ml)	AUC _{0-last} (ng.h/ml)	T _{last} (h)
3 mg/mL 5% DMSO/95% (20% HP-β-CD) in water, clear solution	30	11.6	37.8	1.76
3 mg/mL 10% Transcutol/50% Tween 20/ 40% Maisine, clear solution	30	335	1156	3.58
3 mg/mL 9% PEG-400/ 0.5% Tween-80/ 0.5% PVP-K30/90% CMC- Na (Medium viscosity) in water (0.5%), homogenous opaque suspension	30	81.5	286	2.29

- Log P of this compound is high (> 5)
- SEDDS formulation showed increased systemic exposure, which was probably due to increased gastrointestinal and lymphatic absorption.

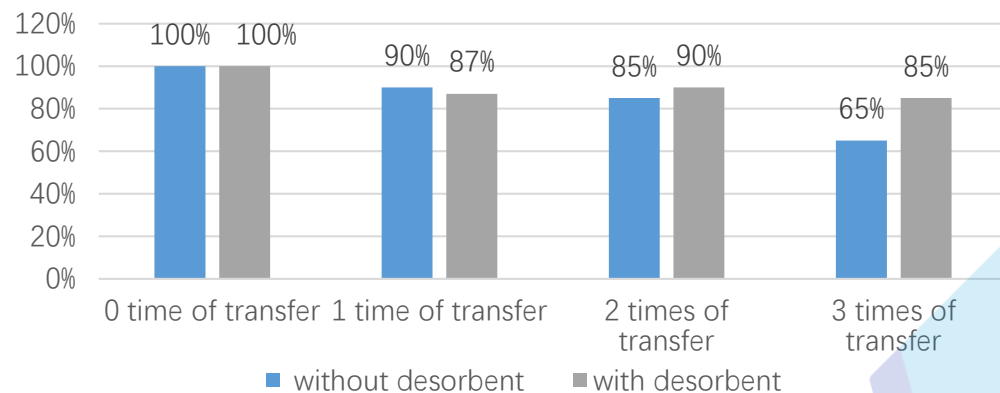
WuXi AppTec DMPK in-house data



ADME-PK Solutions for PROTACs _ Bioanalysis

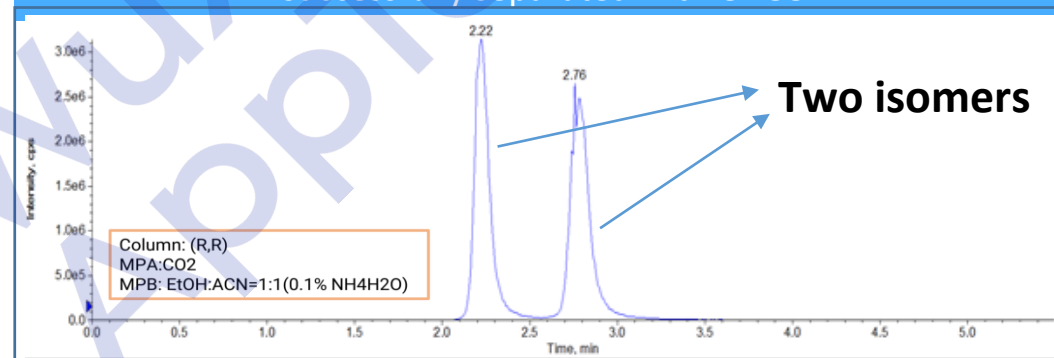
Non-Specific Binding

Plasma Concentrations after Sample Transfer



Multiple Chiral Centers

A PROTAC could not be separated with UPLC but were successfully separated with UPCC



UPCC: Ultra-performance Convergence Chromatography



UPLC-MS/MS



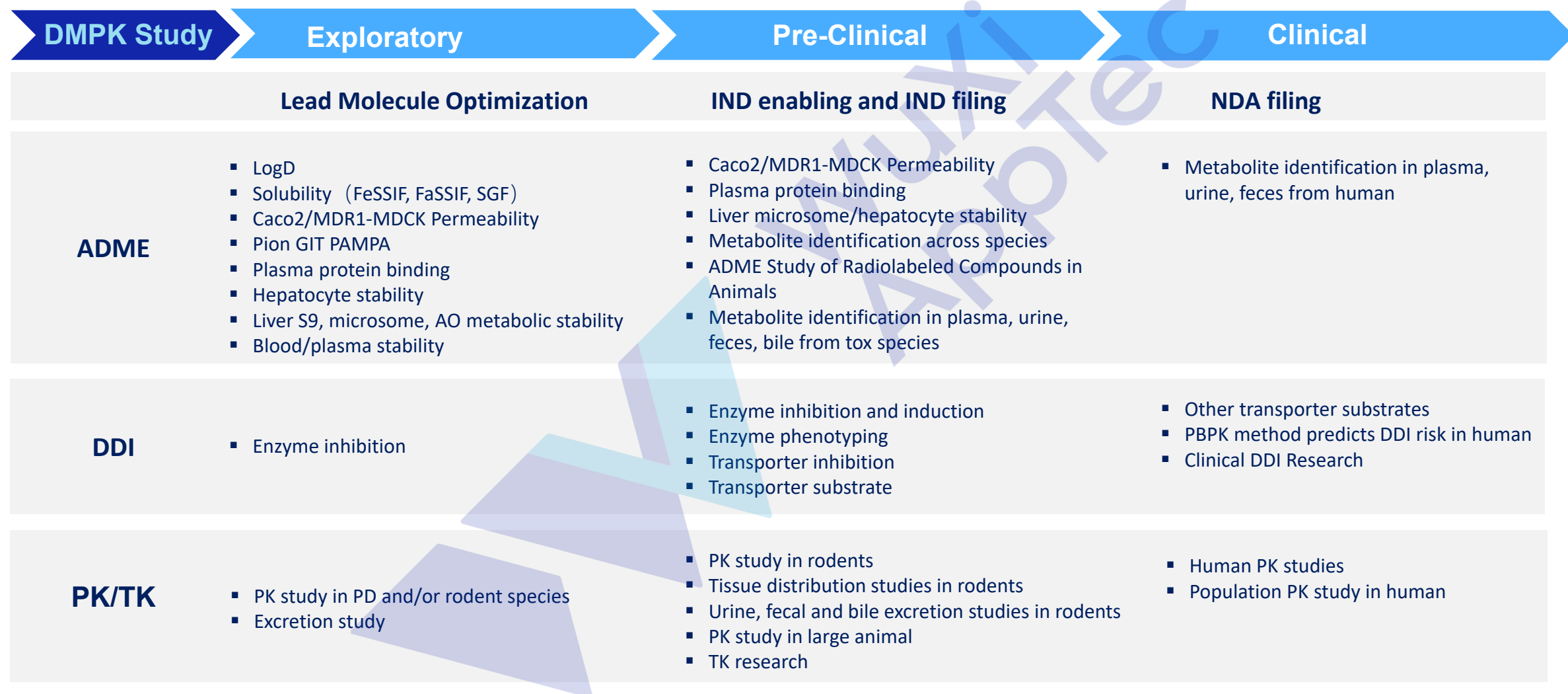
UPCC-MS/MS



Chiral Column Library

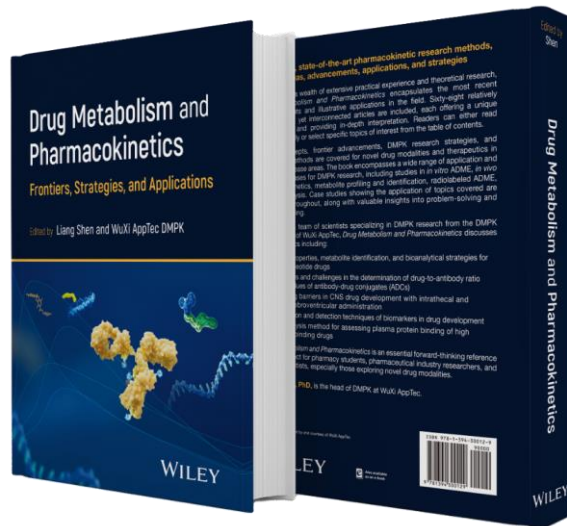
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Strategy and Process of DMPK Studies for PROTACs



Questions

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<https://labtesting.wuxiapptec.com/>

<https://dmpkservice.wuxiapptec.com/>

