



MRC
Toxicology
Unit



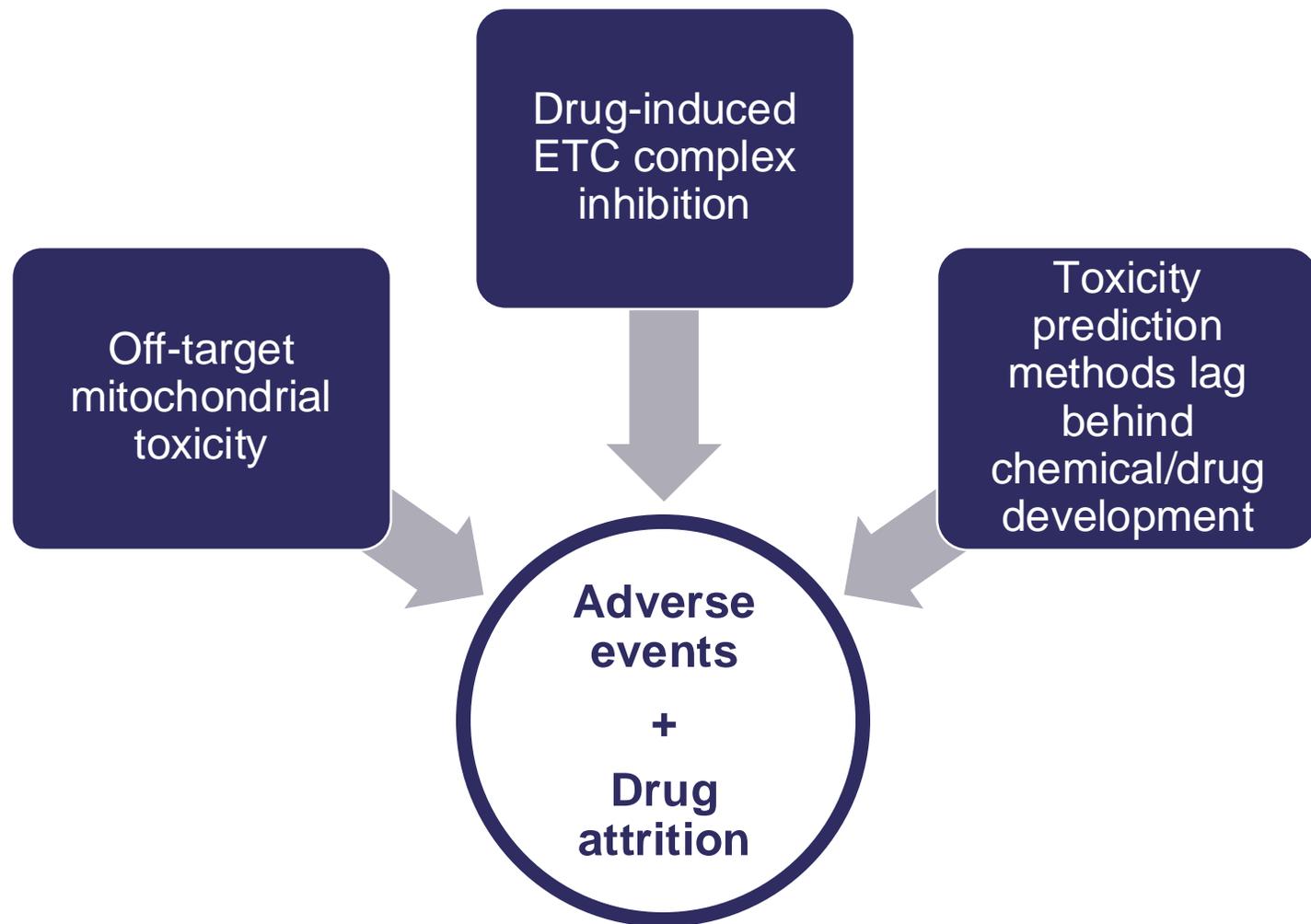
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Active Learning Guides Compound Selection to Improve Mitochondrial Toxicity Screening Efficiency

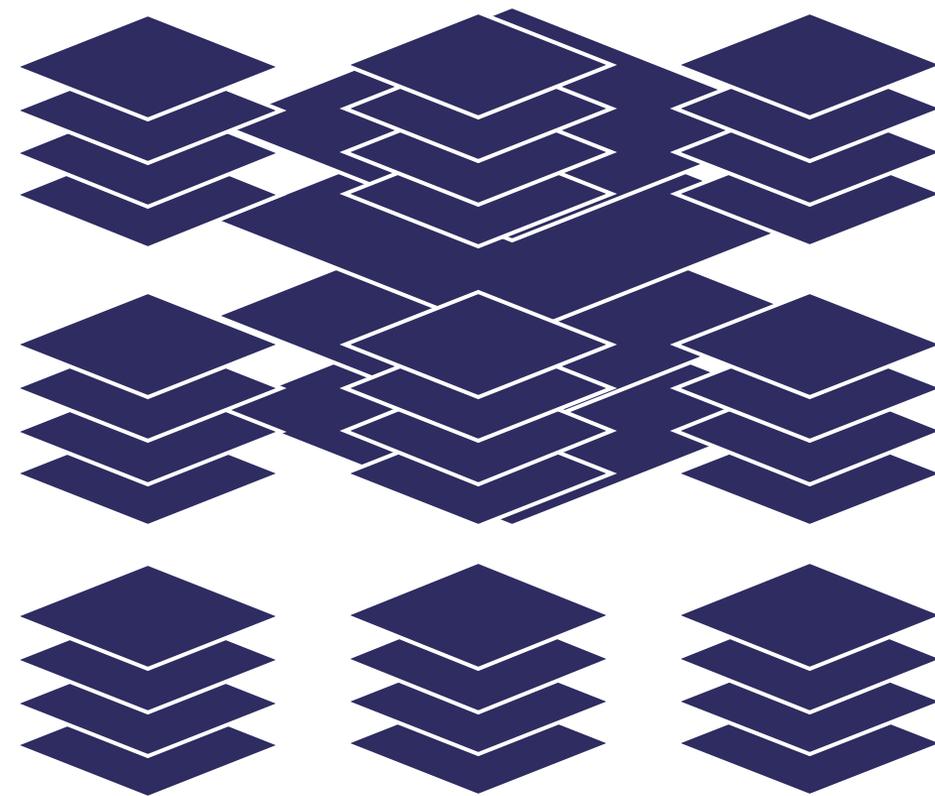
*ASCCT-ESTIV Award Winners Series
Webinar 2024*

Tiago Marques Pedro
22nd November 2024

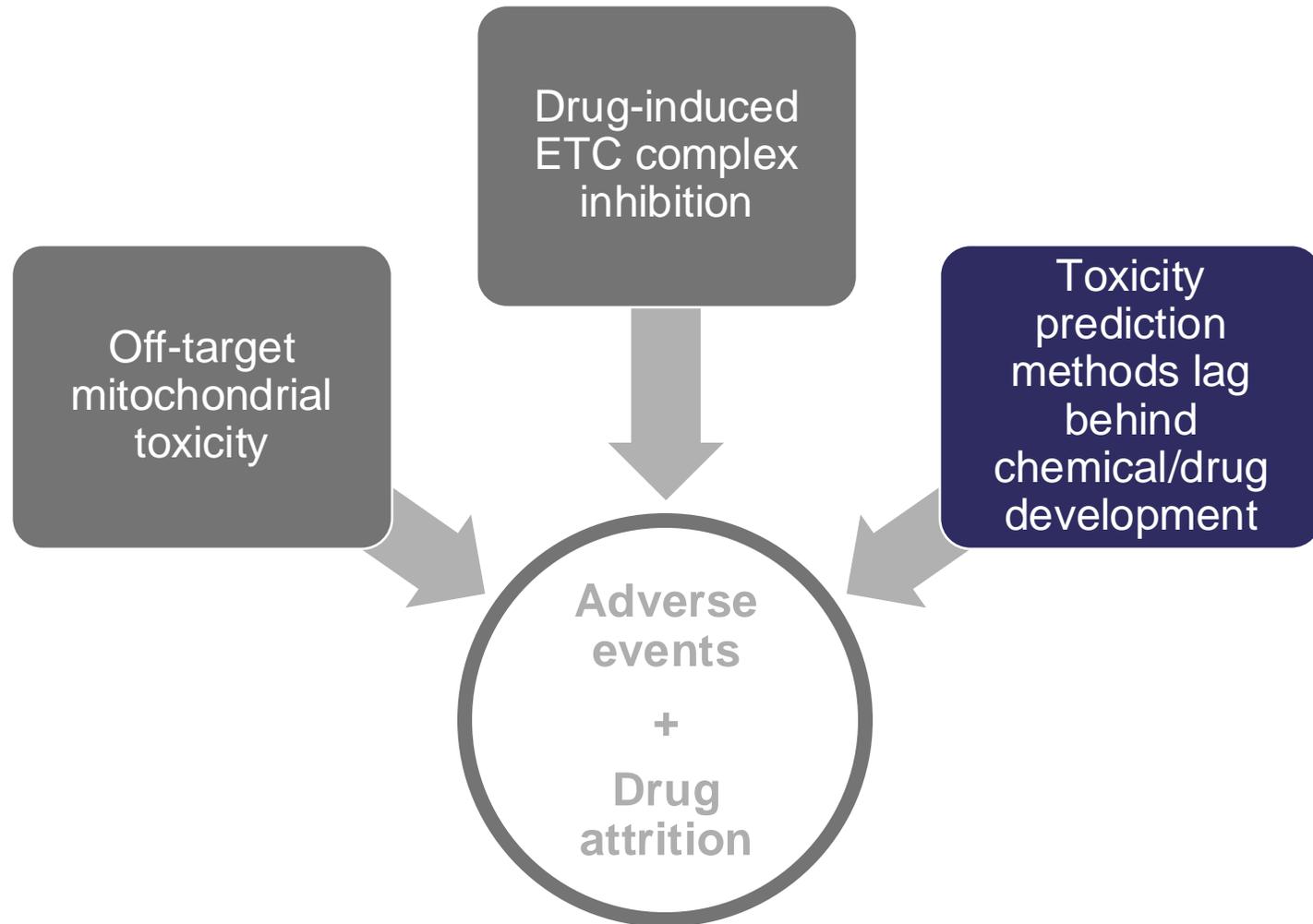
Framing the Problem



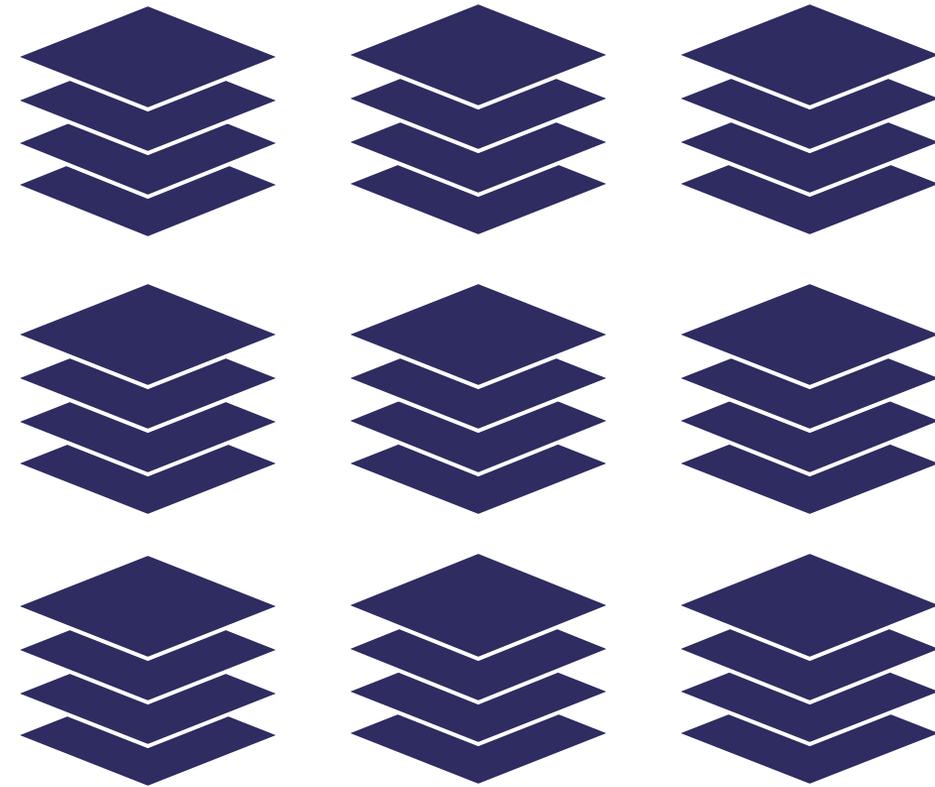
Chemical Library



Mitochondrial Toxicity Screening – Is There a Better Way?



Chemical Library



In Vitro Mitochondrial Toxicity Screening: Prestwick Chemical Library



HepG2 cells cultured in glucose- or galactose-supplemented DMEM (11 mM)

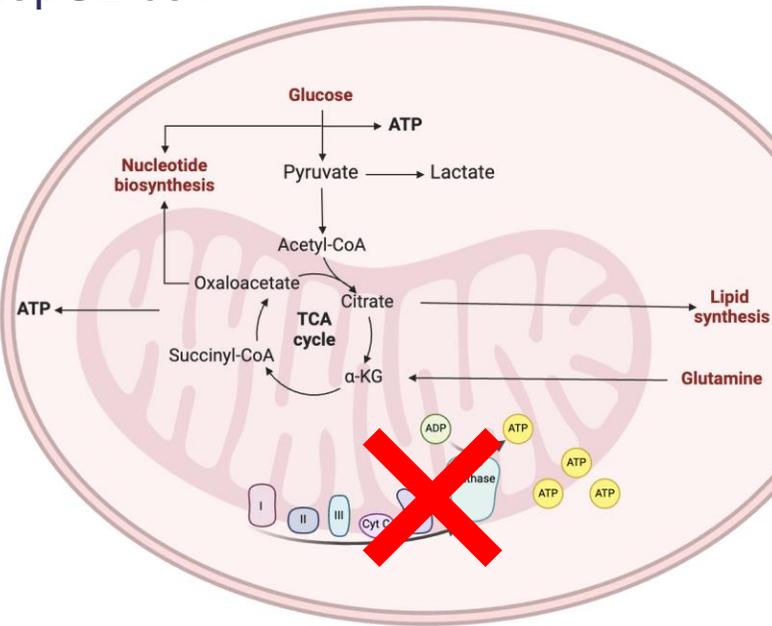
- **7-day switch**
- **20,000 cells** (96-well plate format)
- **50 μM - 18 h treatment**
- Addition of 100 μL of CellTiter Glo Reagent (Promega) – luminescence
- Positive control – Rotenone (50 nM)

Mitochondrial toxicity criteria:

> 30% reduction of cellular ATP in Gal cultured HepG2 cells compared to Glu cells

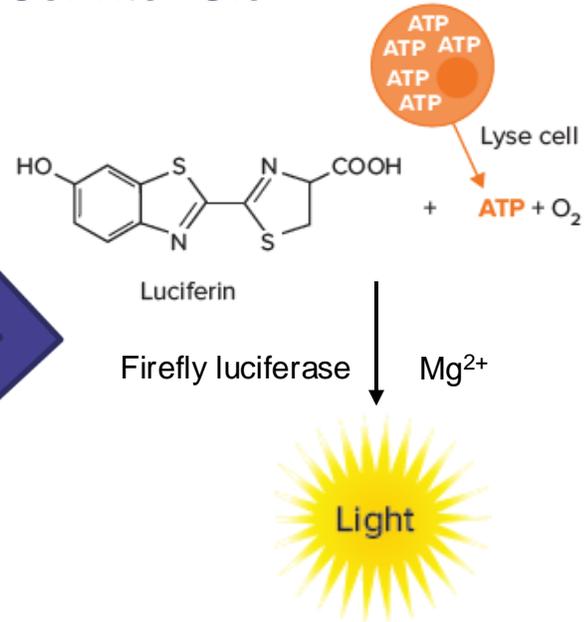
In Vitro Mitochondrial Toxicity Screening: Glu-Gal ATP Assay

HepG2 cell



Glucose conditions

CellTiter Glo

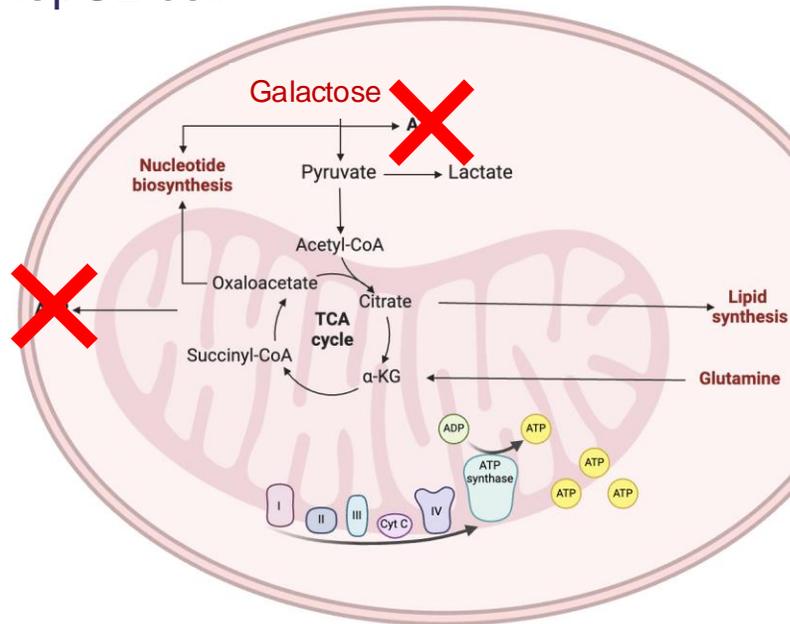


Cellular viability



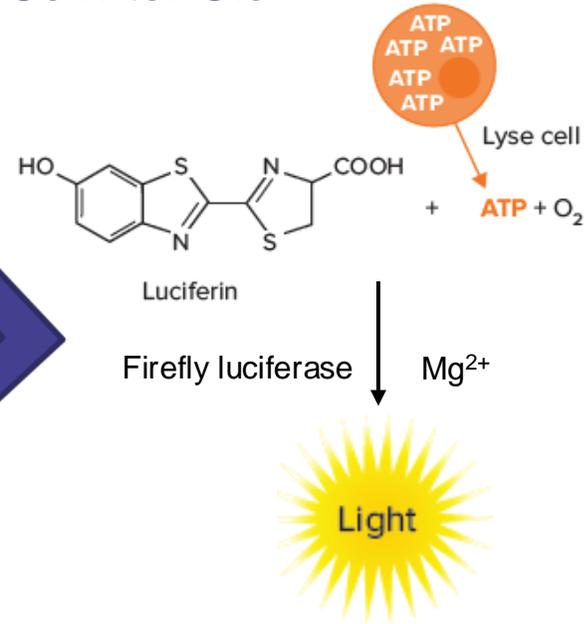
In Vitro Mitochondrial Toxicity Screening: Glu-Gal ATP Assay

HepG2 cell

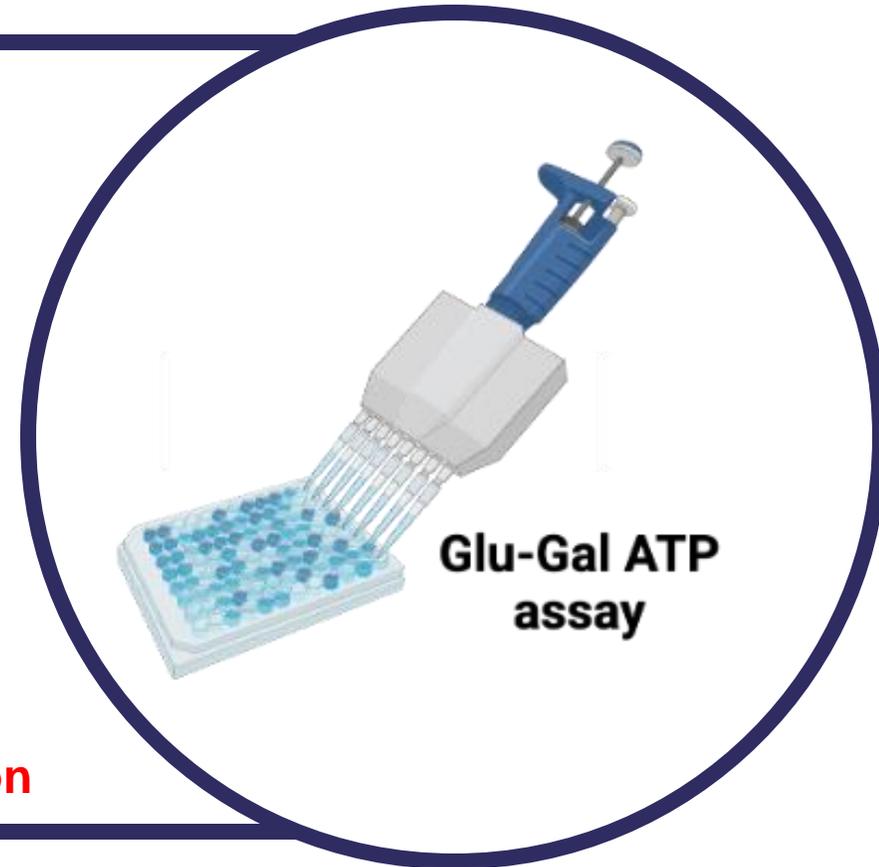


Galactose conditions

CellTiter Glo



Mitochondrial function



In Vitro Mitochondrial Toxicity Screening: Prestwick Chemical Library



**Glu-Gal ATP
assay**

- Glu-Gal HepG2 cells
- 18 h exposure
- 50 μ M

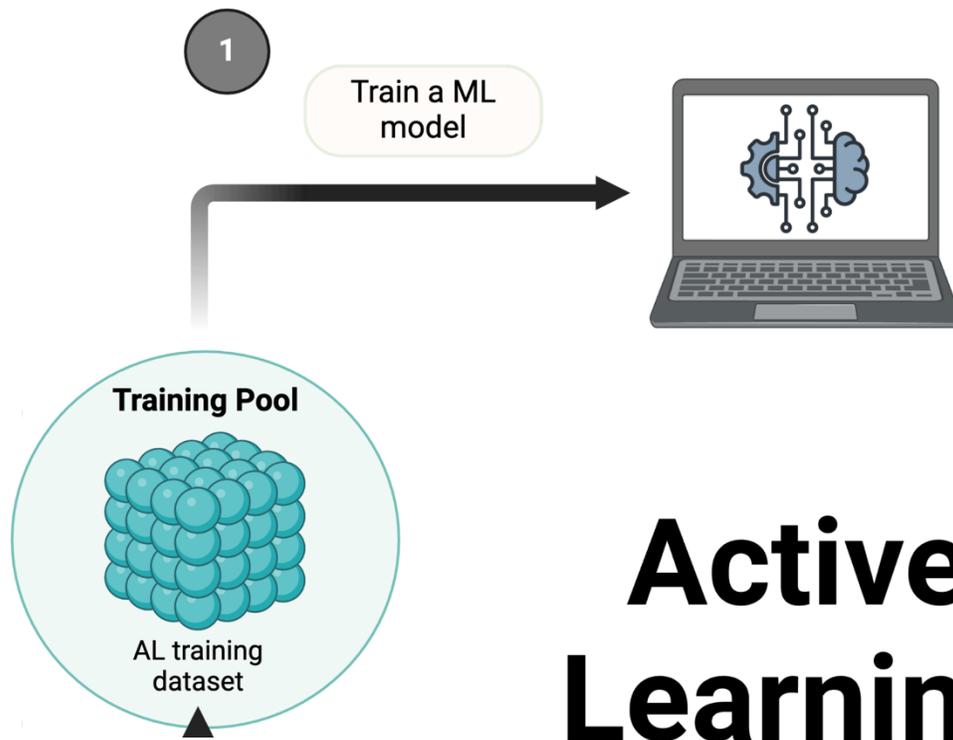


**PRESTWICK
CHEMICAL**

- 1520 small molecule compounds
- 98% FDA and EMA approved drugs
- High chemical diversity



Can we improve the
efficiency of *in vitro*
screening?



Active Learning Workflow

Active Learning Framework – Datasets

		AL training data: <i>Mitochondrial</i>	
		<i>Toxic</i>	<i>Non-toxic</i>
 Tox21 data (MMP)	592	-	
 Approved drugs*	-	566	
		1158	

Mitochondrial toxicity
dataset

TOXIC:

Tox21 Mitochondrial Membrane Potential

- HepG2 cells
- Mito-MPS dye + CellTiter Glo
- >8,000 compounds tested
- >1,000 “Actives” → *Mitochondrial Toxic*

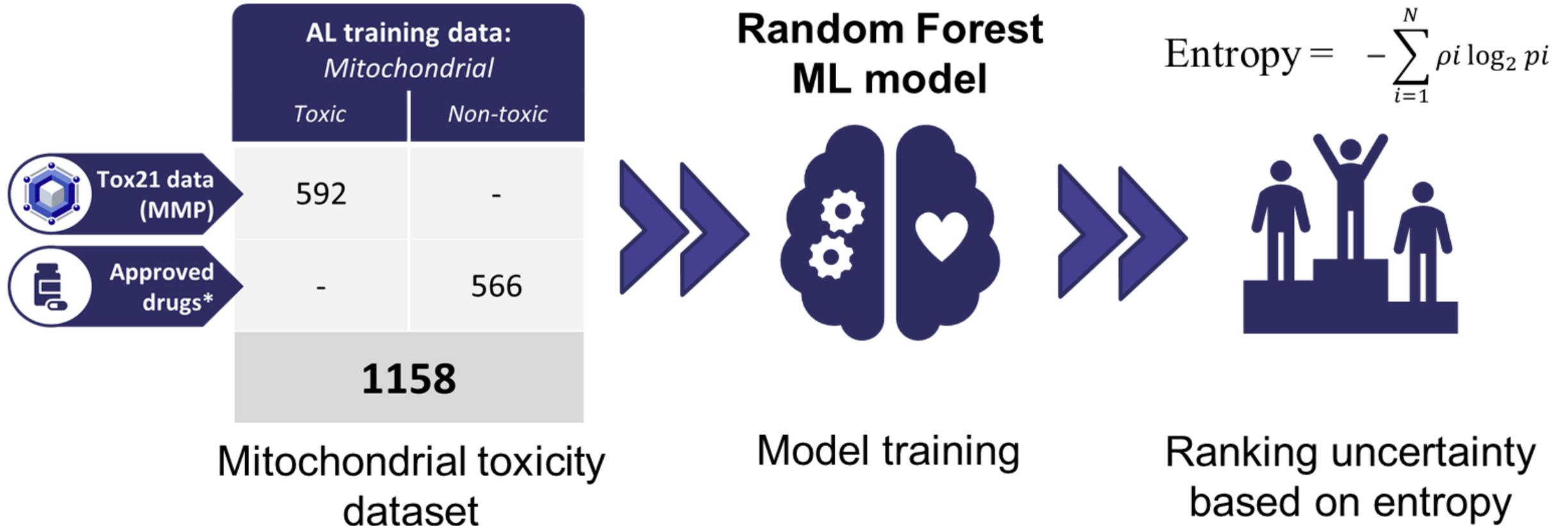
NON-TOXIC:

*Approved drugs dataset

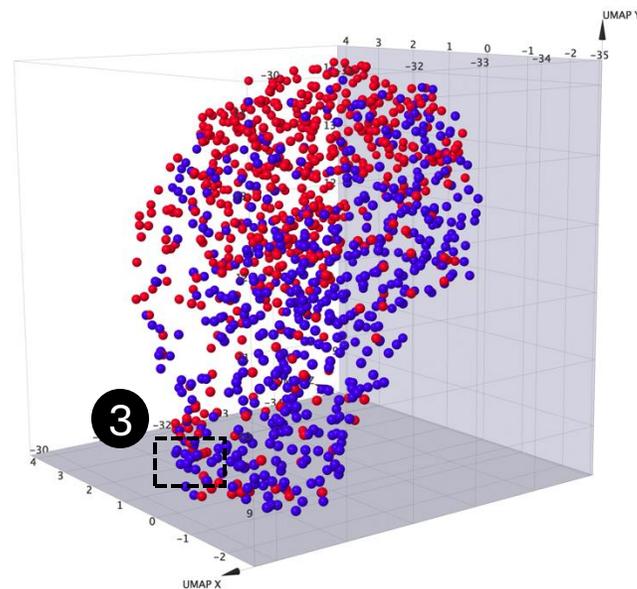
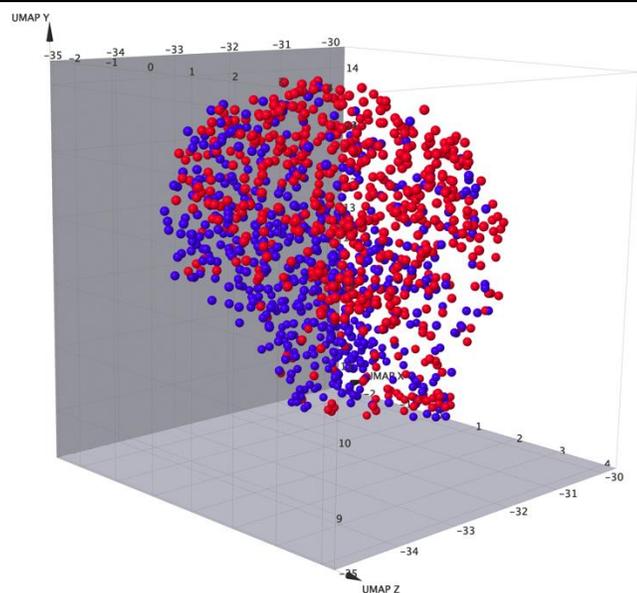
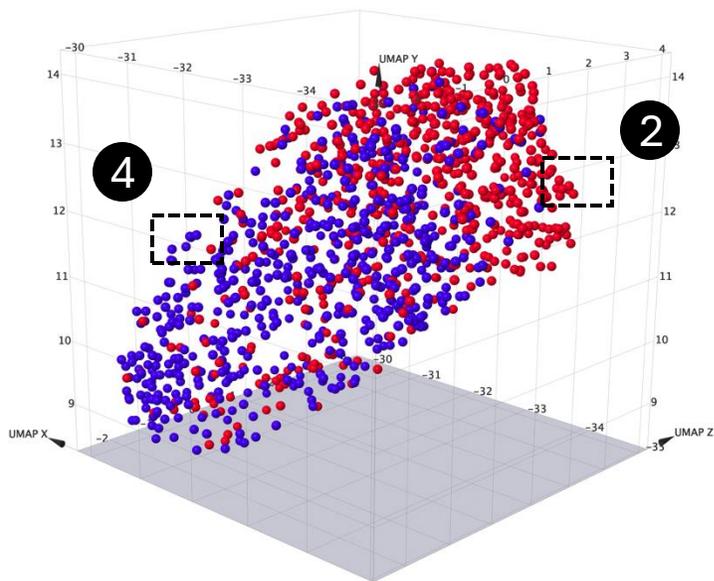
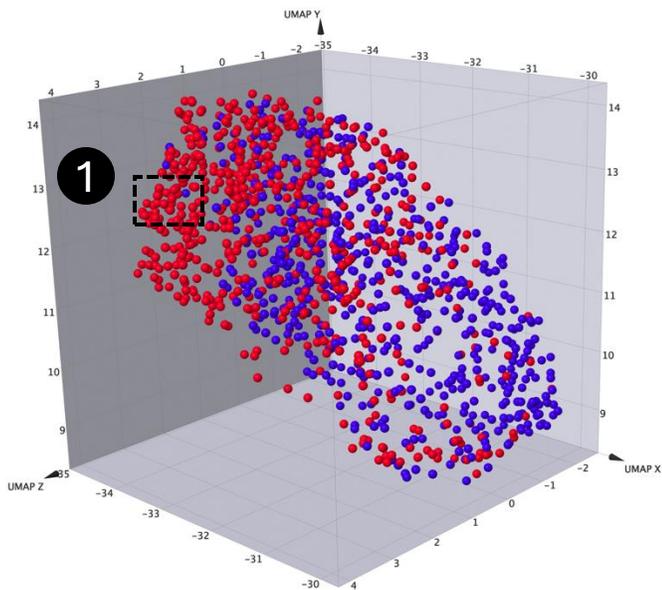
- ChEMBL database
- Drug compounds that have been approved and released on the market
- No mitochondrial mechanism of action
- Hepatotox and Cardiotox alerts removed
- No chemicals with literature evidence of mitochondrial toxicity

Further data processing was conducted to handle duplicates and ensure a chemically diverse dataset

Active Learning Framework



Comparison of Toxic vs Non-toxic Chemicals in the AL Training Data



AL Mitotox Labels



Toxic

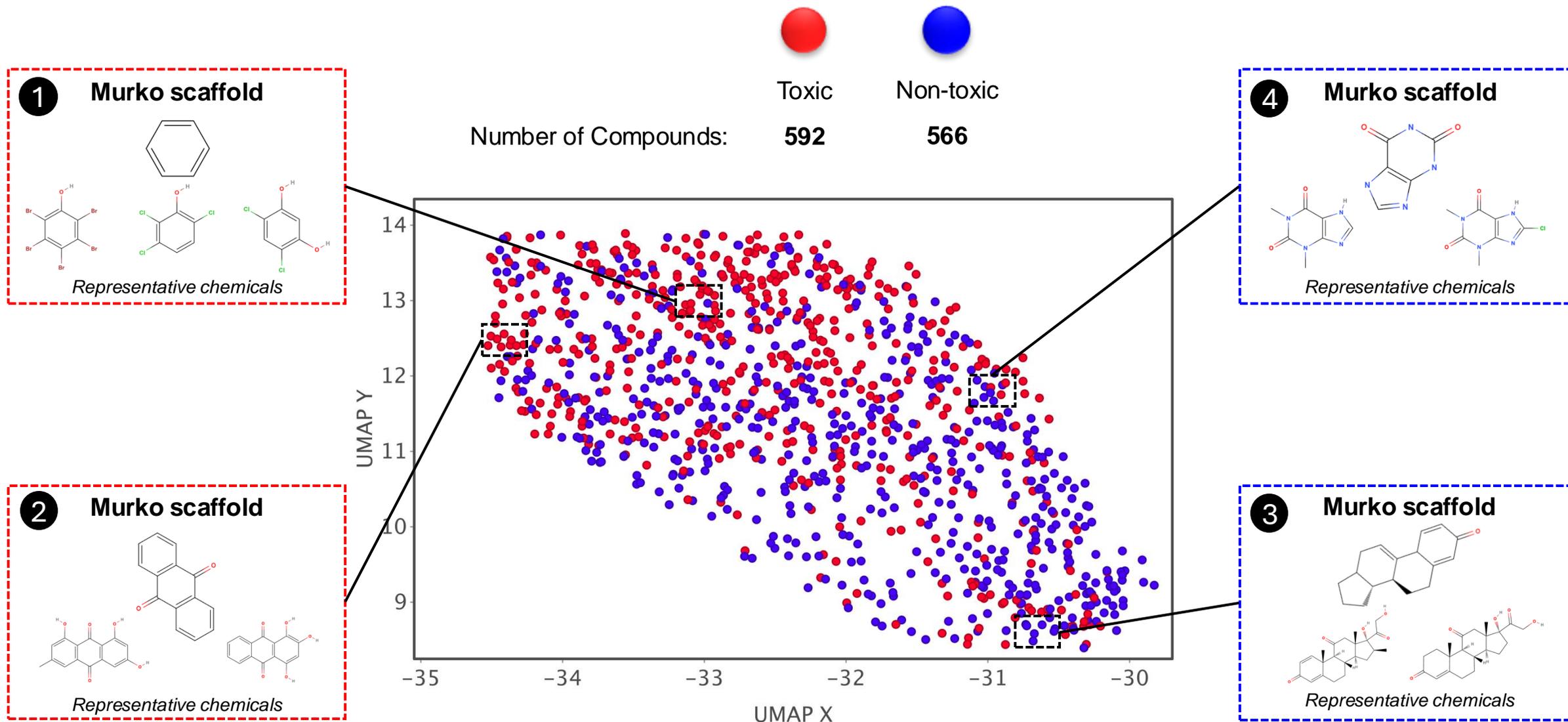
Non-toxic

Number of
Compounds:

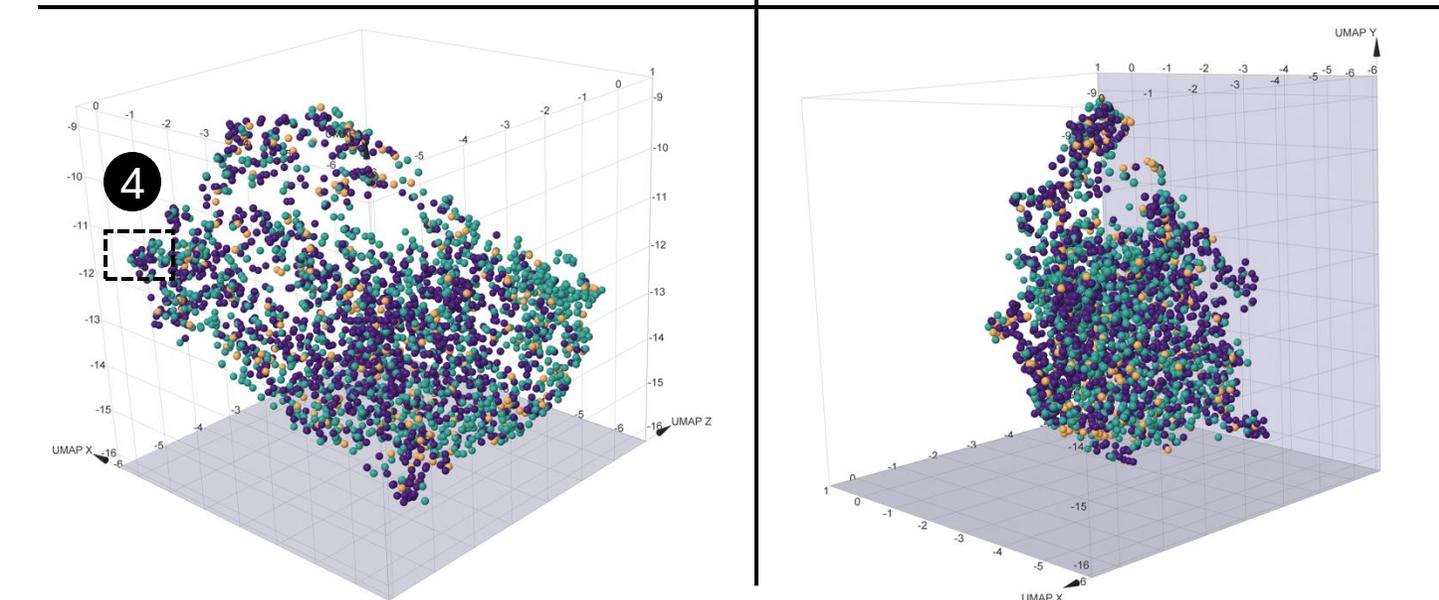
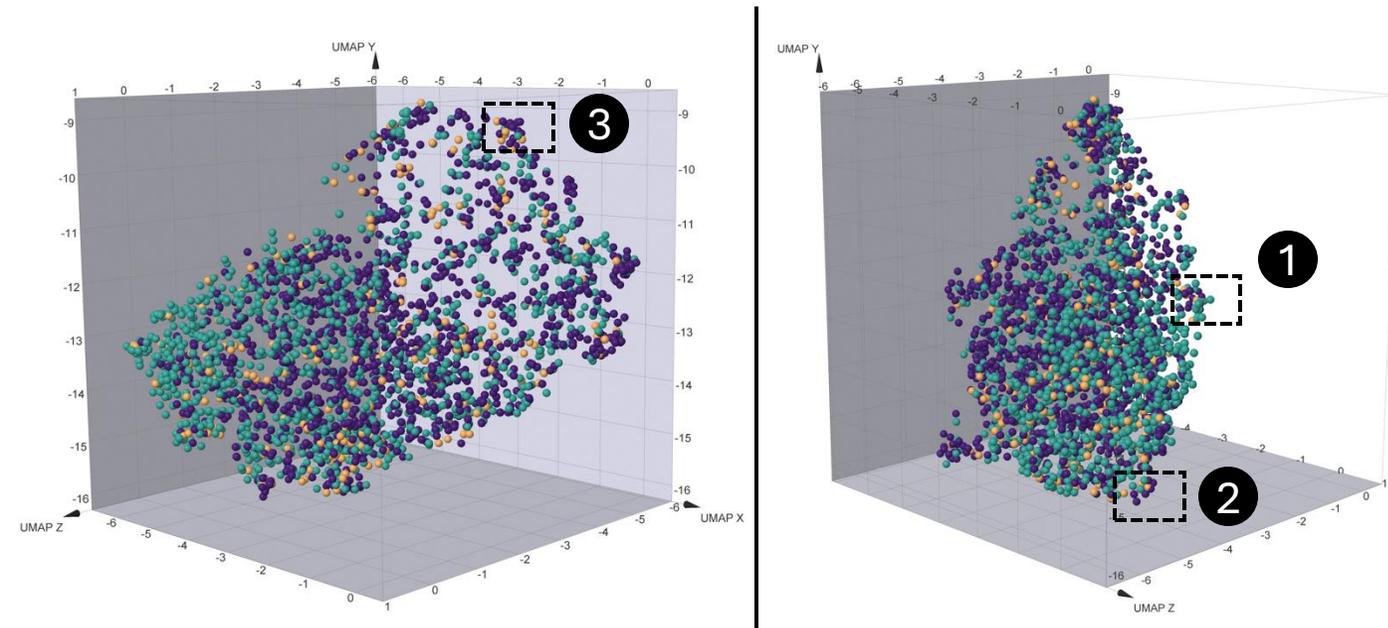
592

566

Comparison of Toxic vs Non-toxic Chemicals in the AL Training Data



Comparison of AL Training Data and Prestwick Library



	Library		
			
	AL training data	Prestwick	Both
Number of Compounds:	1158	1510	255

Comparison of AL Training Data and Prestwick Library



AL training
data

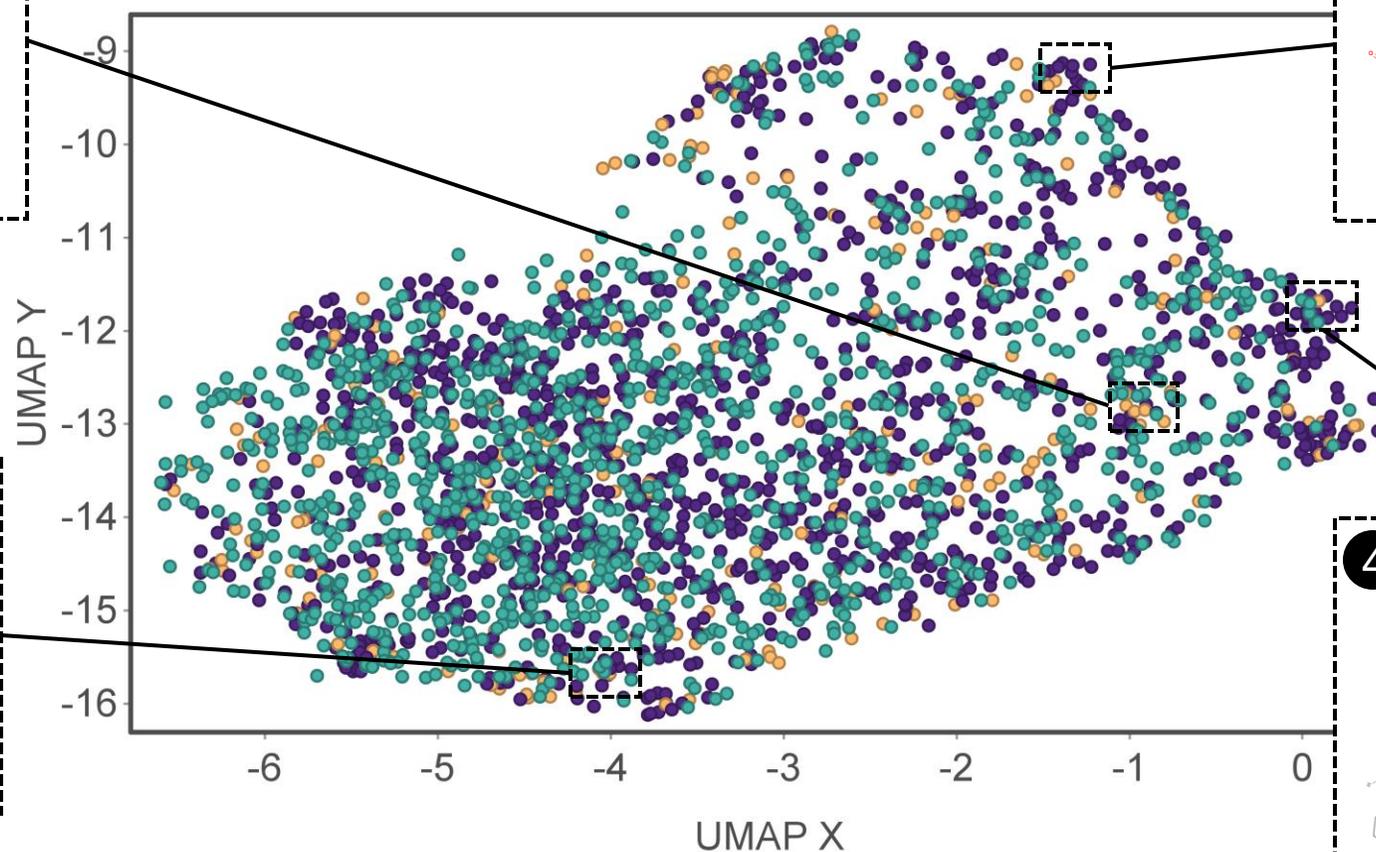
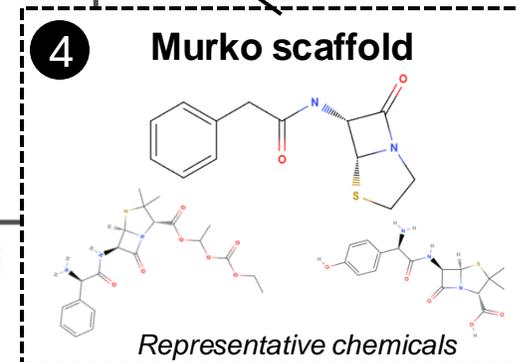
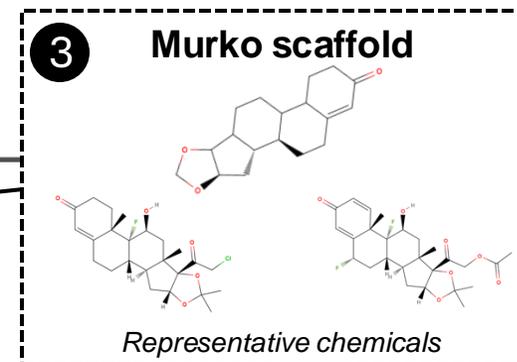
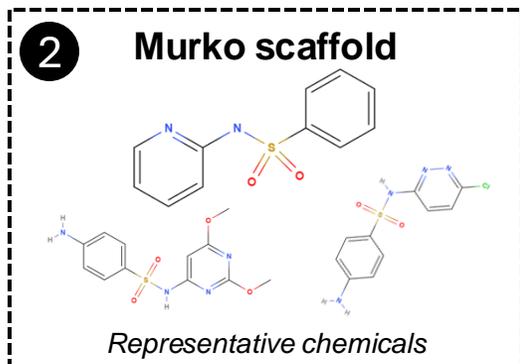
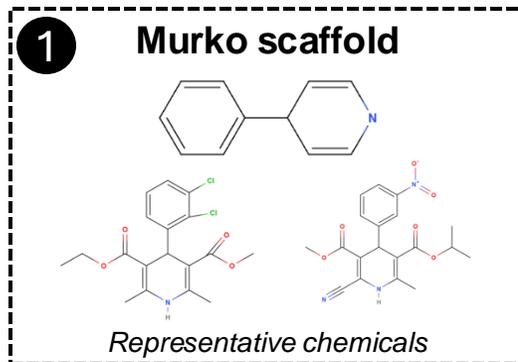
Prestwick

Both

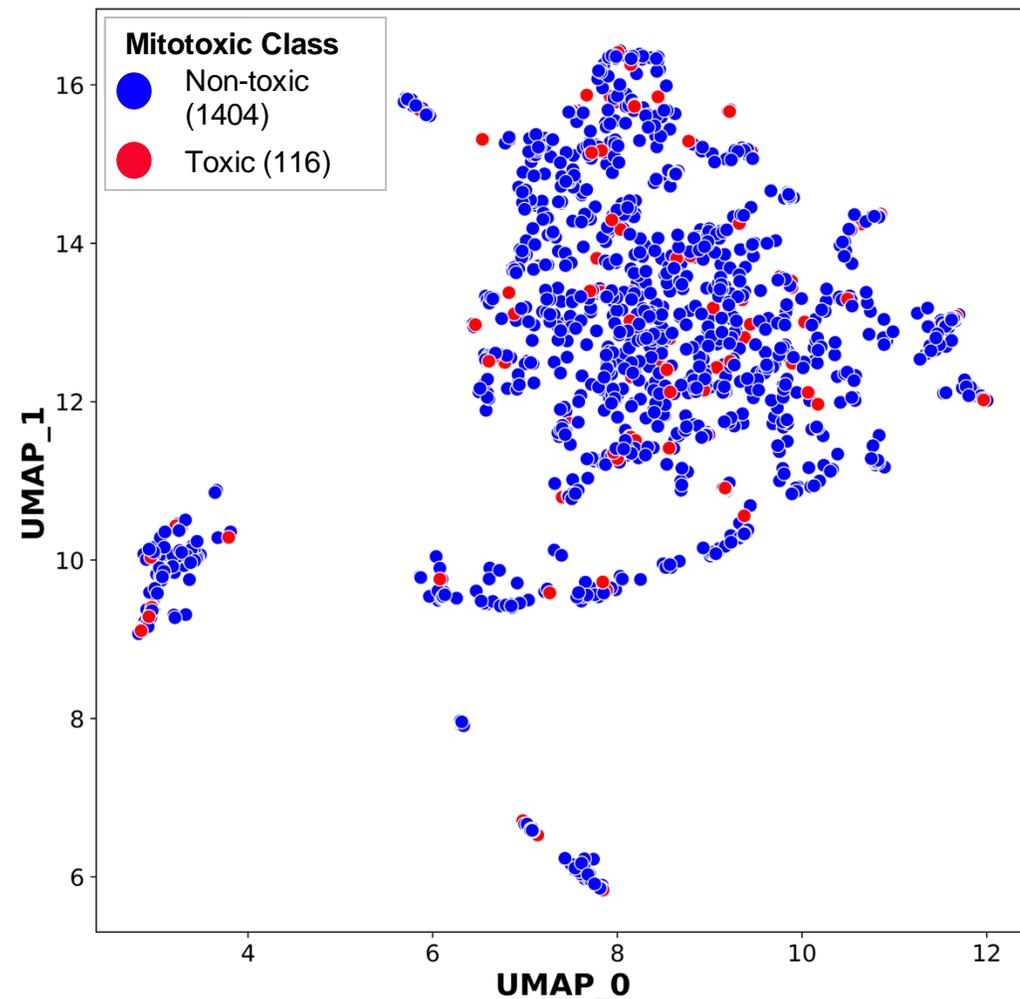
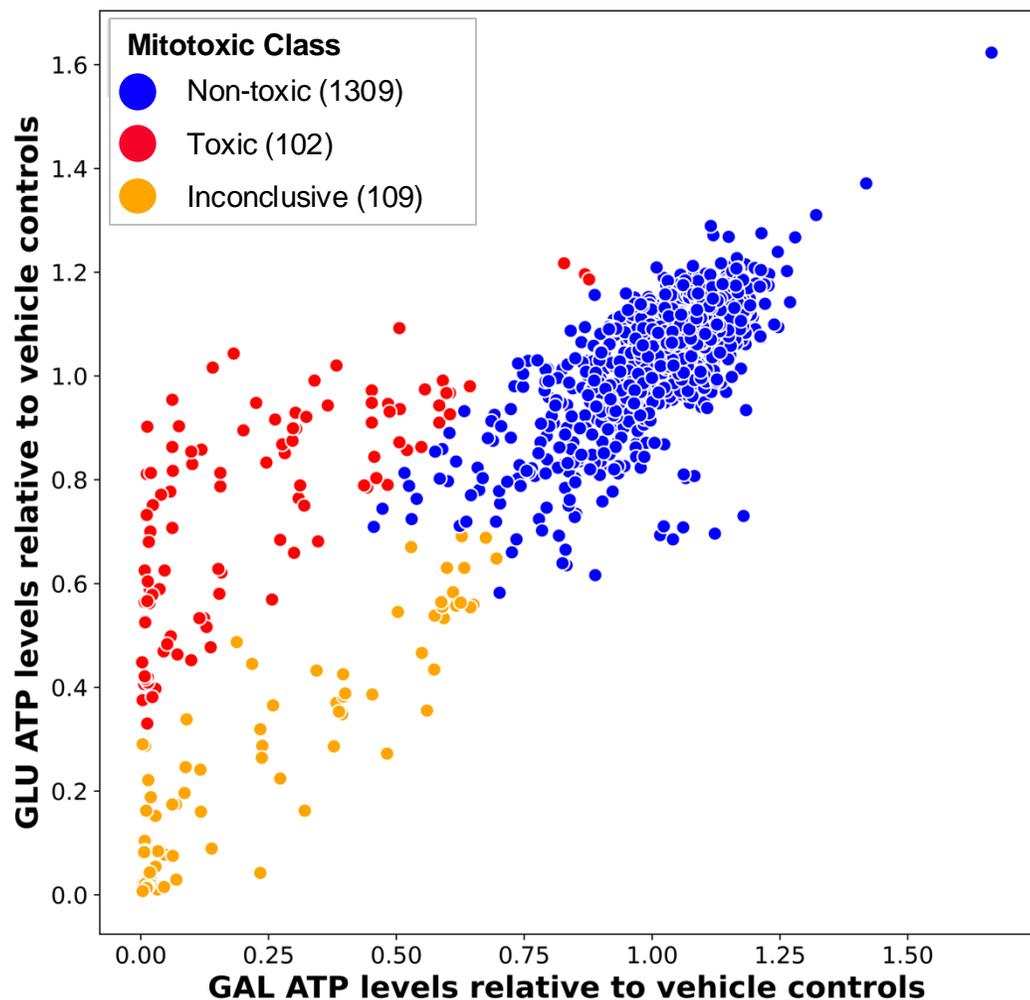
Number of Compounds: **1158**

1510

255



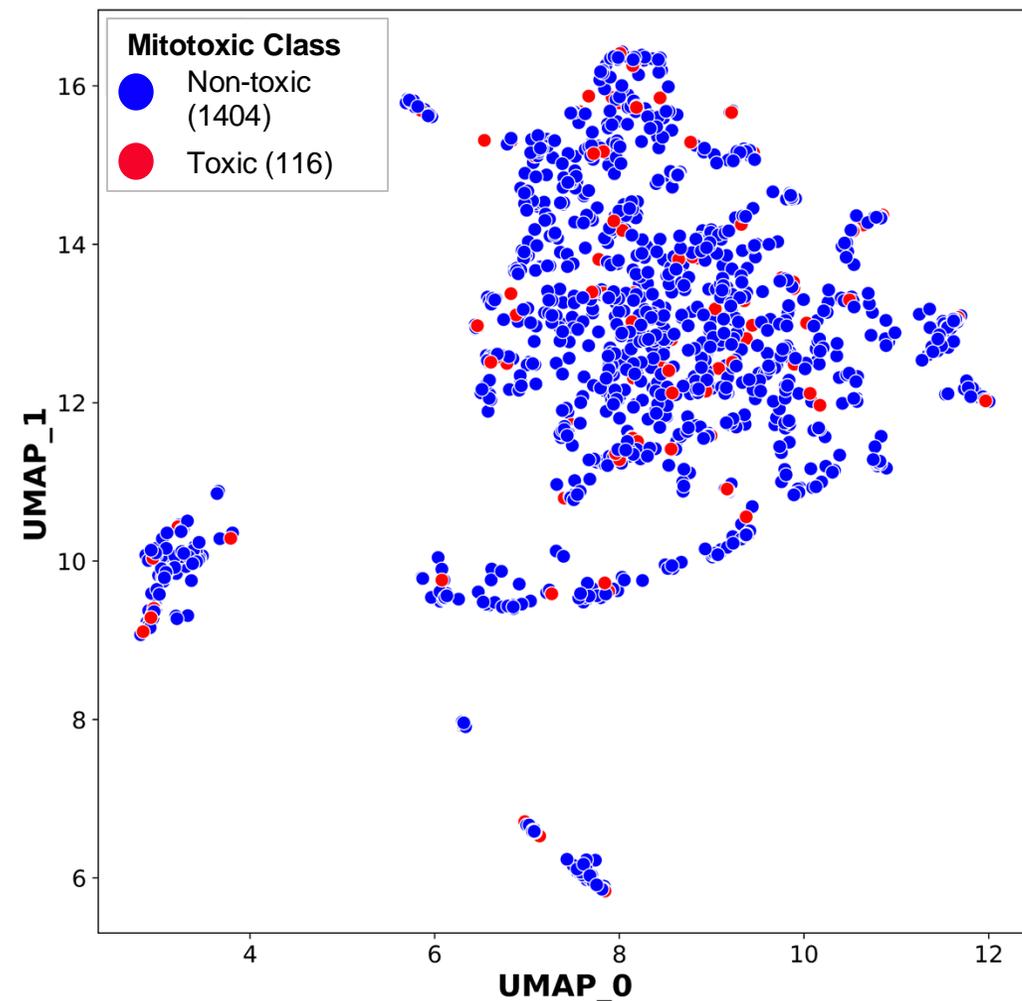
Prestwick Chemical Library Screening – Mitotoxic Class Visualised Based on ATP Levels and Structure



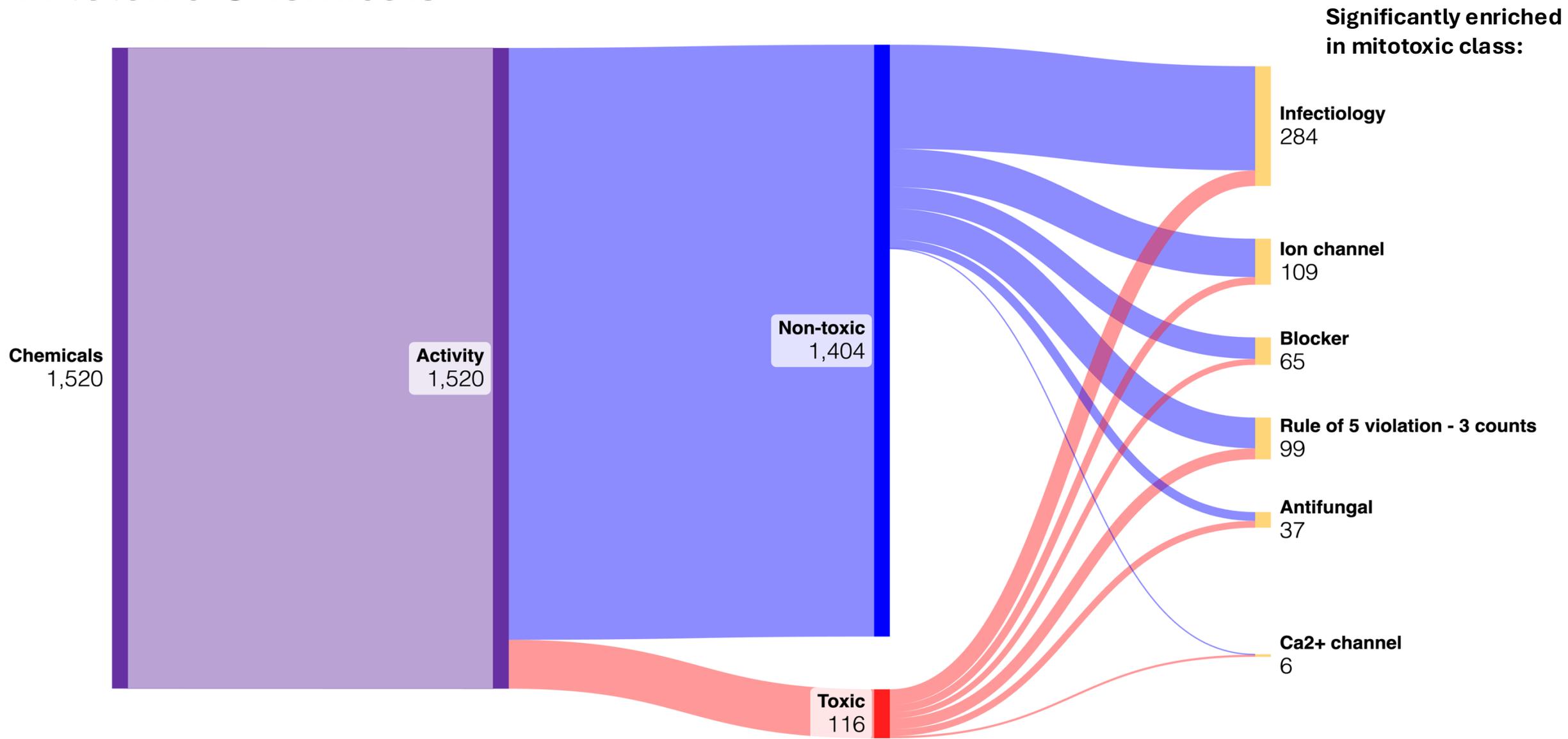
Prestwick Chemical Library Screening – Activity Comparison of the Same Chemicals in the Prestwick Chemical Library and Tox21

Chemical overlap of Prestwick and Tox21 libraries comparing activity labels for their respective assays (Glu-Gal ATP vs MMP)

		MMP	
		Toxic	Non-toxic
Glu-Gal ATP	Toxic	35	0
	Non-toxic	0	503

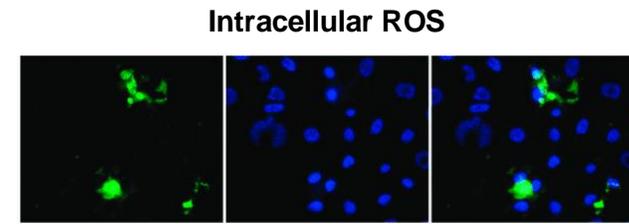


Prestwick Chemical Library Screening – Descriptor Enrichment in Mitotoxic Chemicals

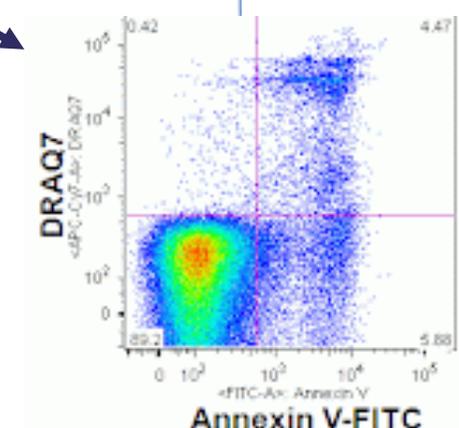
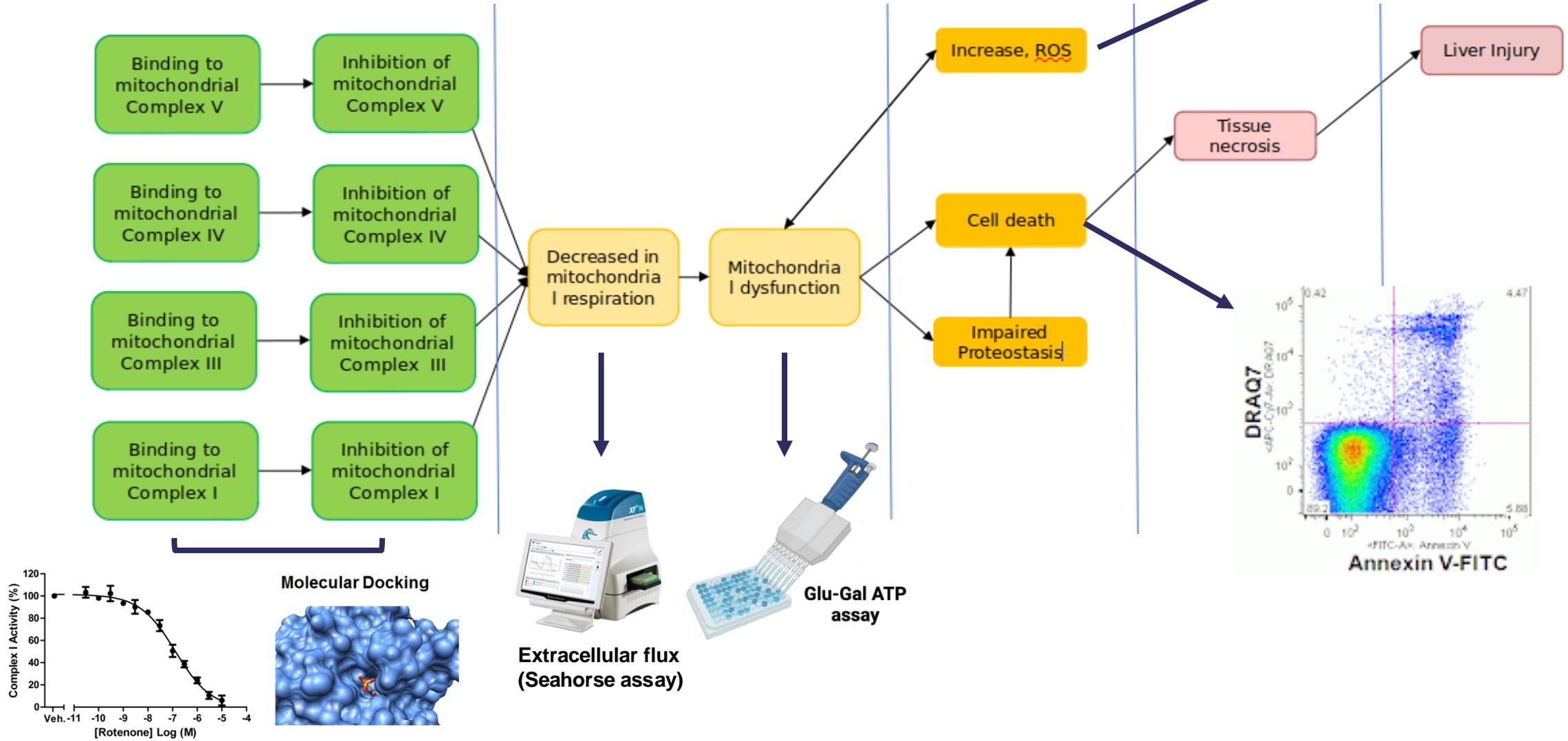


Mechanistic Investigation of Mitotoxic Hits

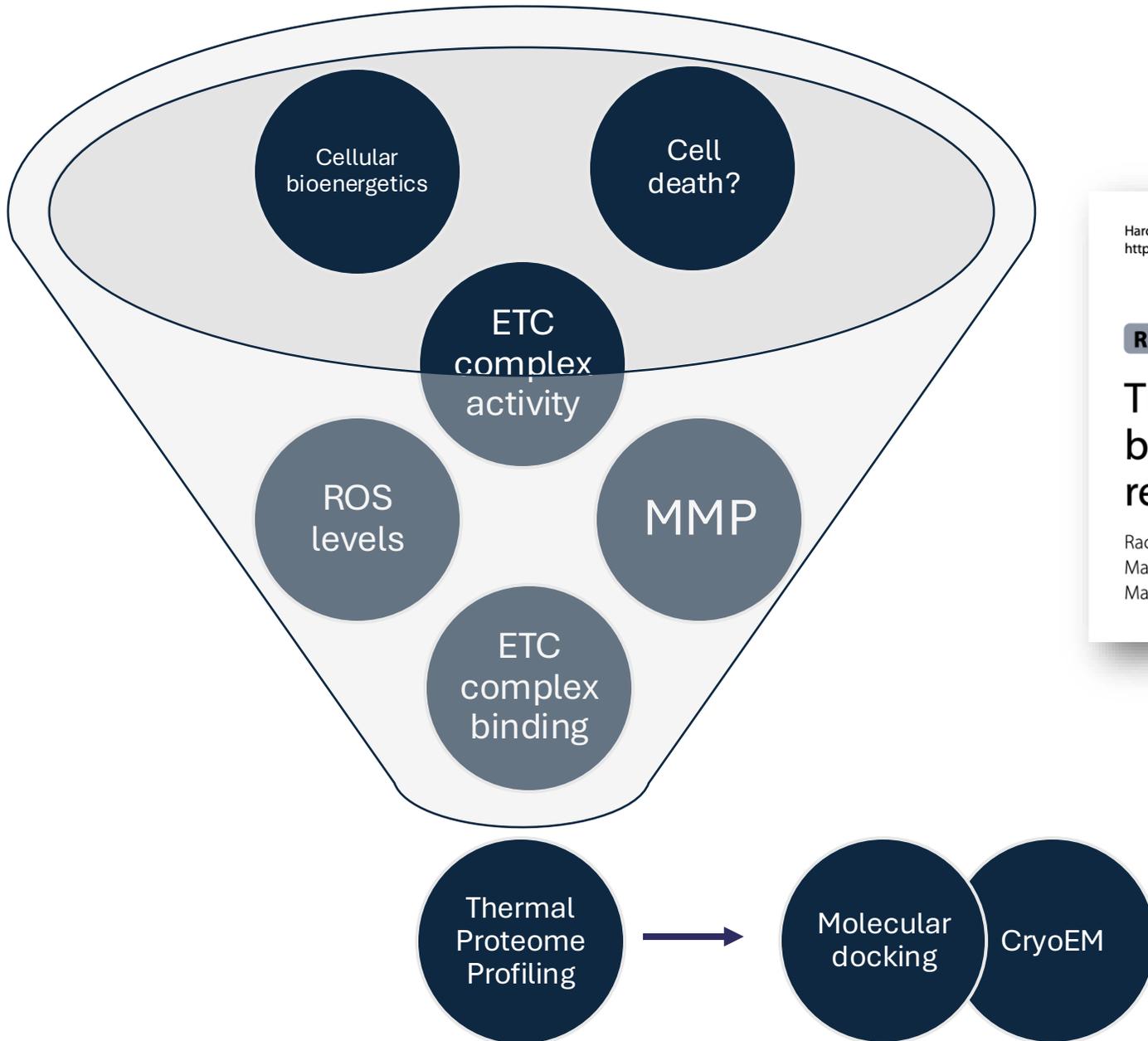
AOP: 273 - Mitochondrial complex inhibition leading to liver injury



Intracellular ROS



Mechanistic Investigation of Mitotoxic Hits



Hardy et al. *Biology Direct* (2023) 18:43
<https://doi.org/10.1186/s13062-023-00375-9>

Biology Direct

RESEARCH

Open Access

The antipsychotic medications aripiprazole, brexpiprazole and cariprazine are off-target respiratory chain complex I inhibitors



Rachel E. Hardy¹, Injae Chung², Yizhou Yu¹, Samantha H. Y. Loh¹, Nobuhiro Morone¹, Clement Soleilhavoup¹, Marco Travaglio¹, Riccardo Serrelli², Lia Panman¹, Kelvin Cain¹, Judy Hirst², Luis M. Martins^{1*}, Marion MacFarlane^{1*} and Kenneth R. Pryde^{1*}



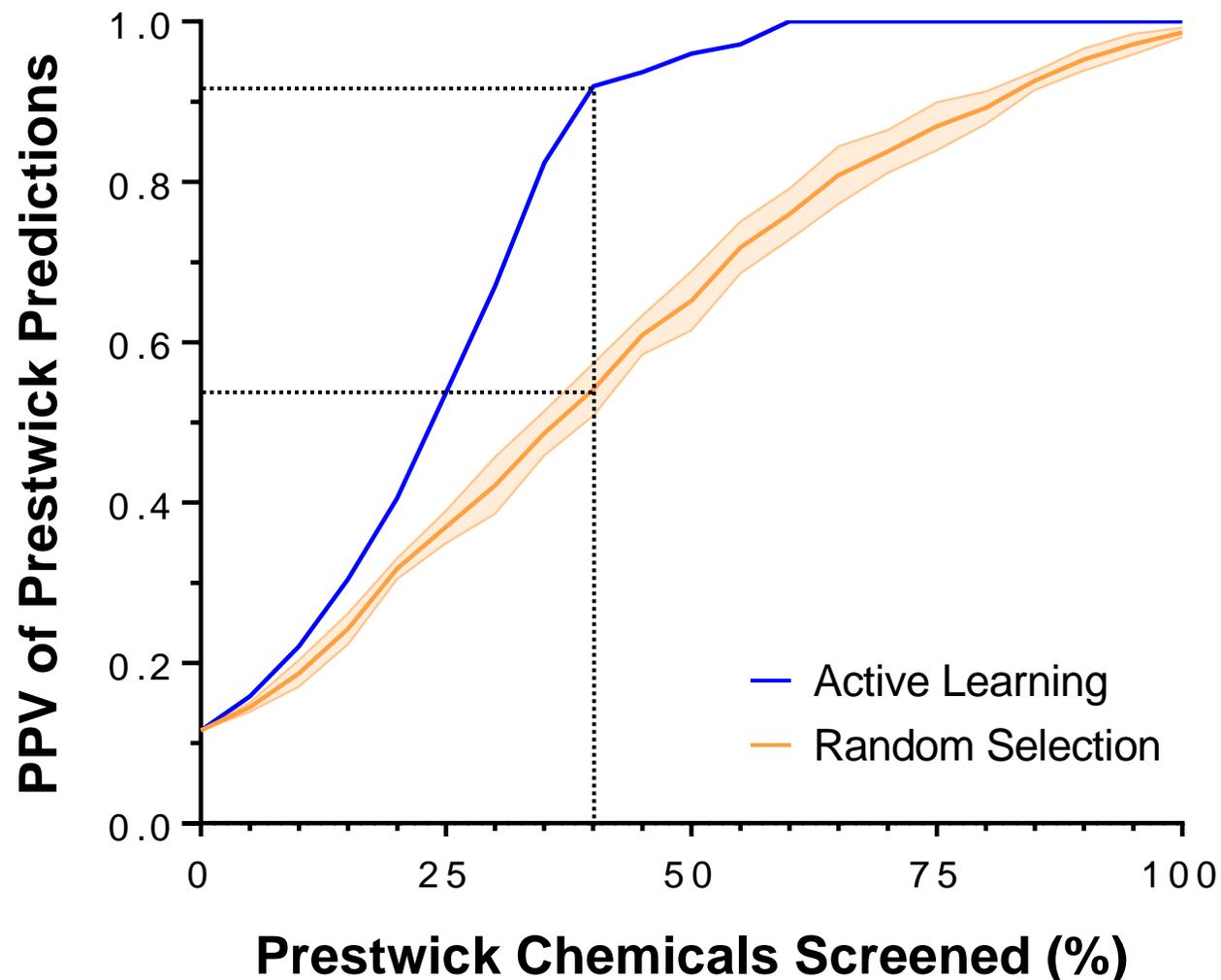
Can Active Learning improve the efficiency of *in vitro* screening?

Active Learning Improves Identification of Mitotoxic Chemicals

Random selection = “traditional” screening methods

AL exhibited a PPV of 0.92 compared to 0.61 when only 40% of the library was screened

Positive Predictive Value (PPV)



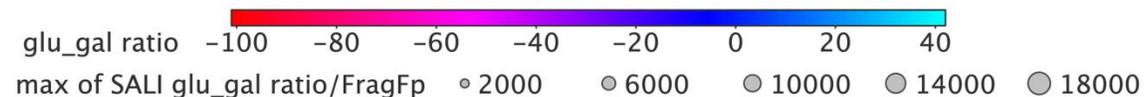
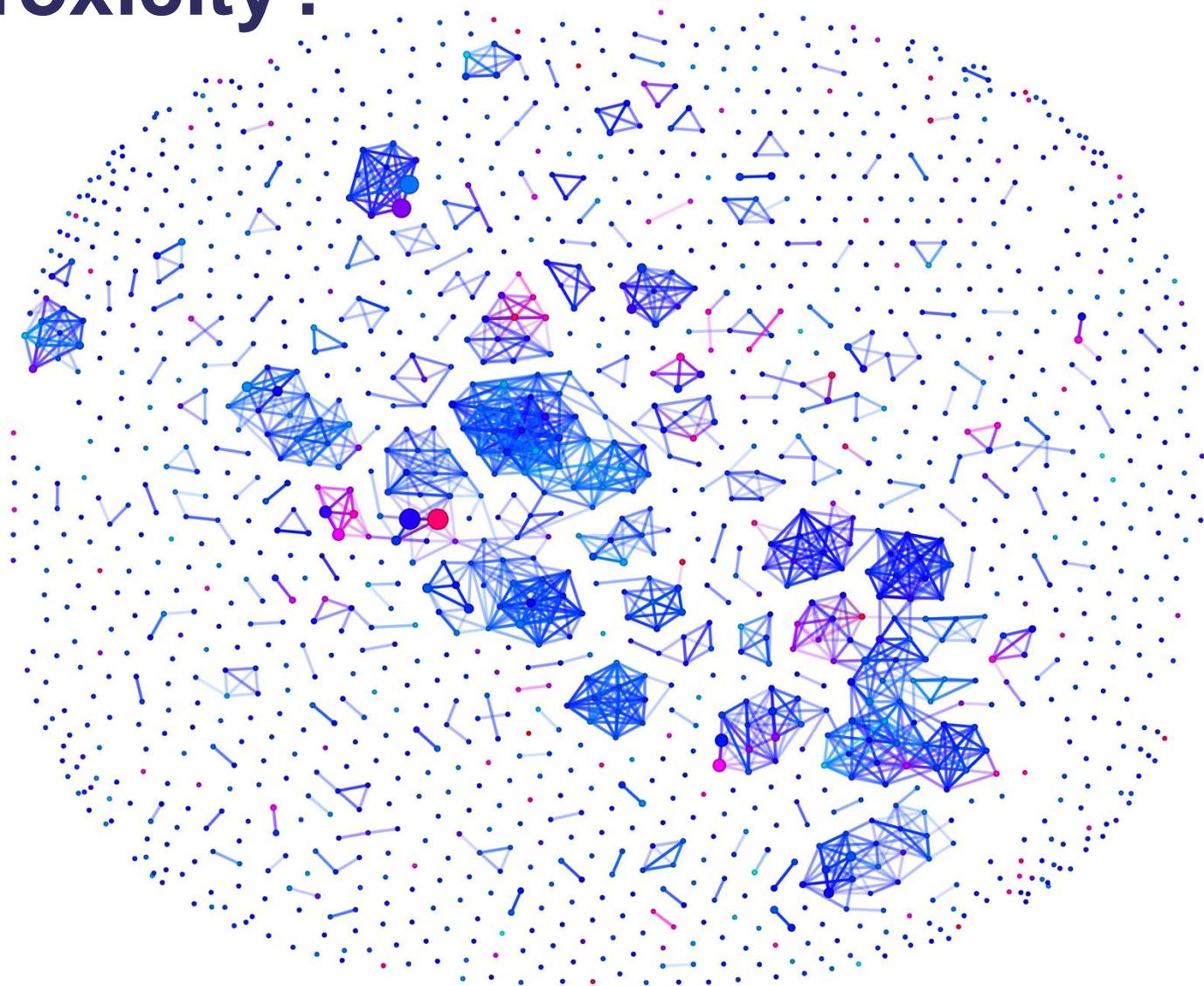
Does Structure Drive Toxicity?

Prestwick Chemical Library Glu-Gal
ATP Screening Results:

116 mitotoxic chemicals

?

Can we identify structural differences driving toxicity?

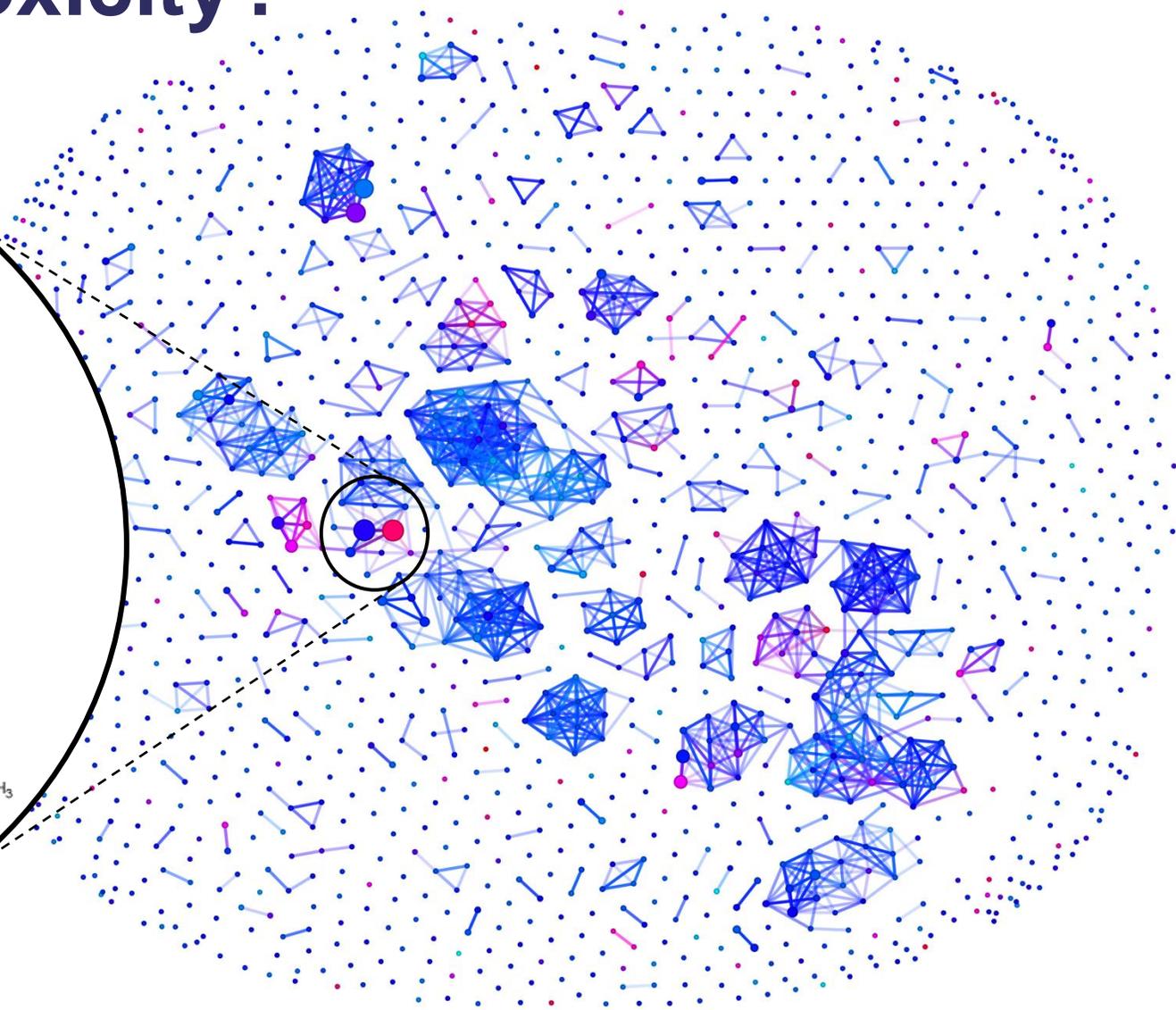
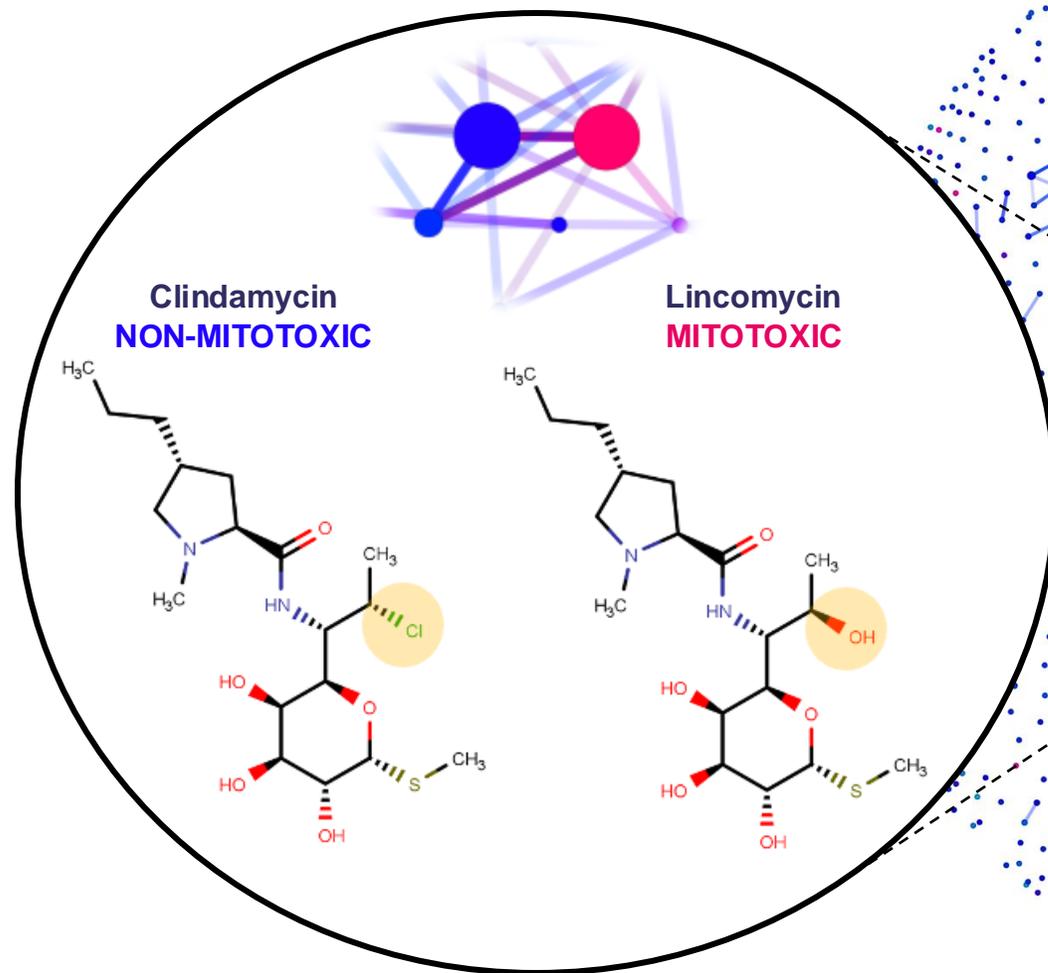


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Does Structure Drive Toxicity?

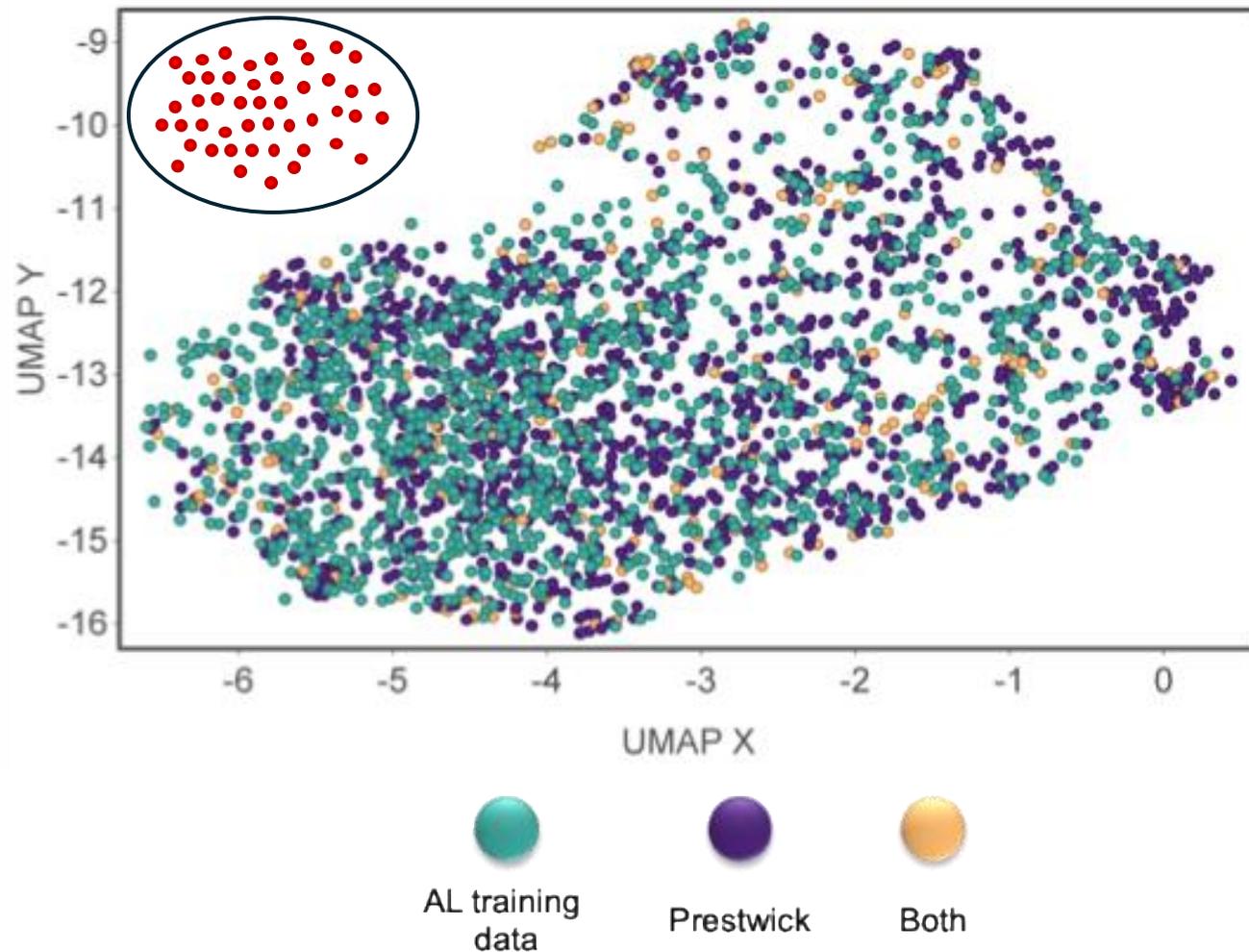


Summary

- Active learning was able to predict mitotoxic compounds with >90% accuracy when screening less than half of the library
- This framework benefits resource limited situations or when the identification of toxic compounds must be prioritised



How well does the model extrapolate to unseen data?



Acknowledgements

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Martins Lab

Miguel Martins*

Yizhou Yu

Patil Lab

Kiran Patil

Nonantzin Beristain

Stephan Kamrad

Anna Lindell

Bender Lab

Andreas Bender

