Network Analysis and Graph Deep Learning for Investigating Drug Toxicity

February 2024

Sun Kim

Seoul National University

Mogam Institute for Biomedical Research

AIGEN**DRUG** Co. Ltd

Agenda

- Network analysis and deep graph learning for drug discovery

  - Chemical space:
    - DILI toxicophores (patient level data)
    - Modeling substructures (bioassay data)
    - Modeling multiple bioassay data simultaneously for toxicity
    - Side effects of drugs (patient level data)

  - Genetic space:
    - Degree of toxicity (cell line data)

Chemical Space

Toxicophore
Substructures of drug related to toxicity

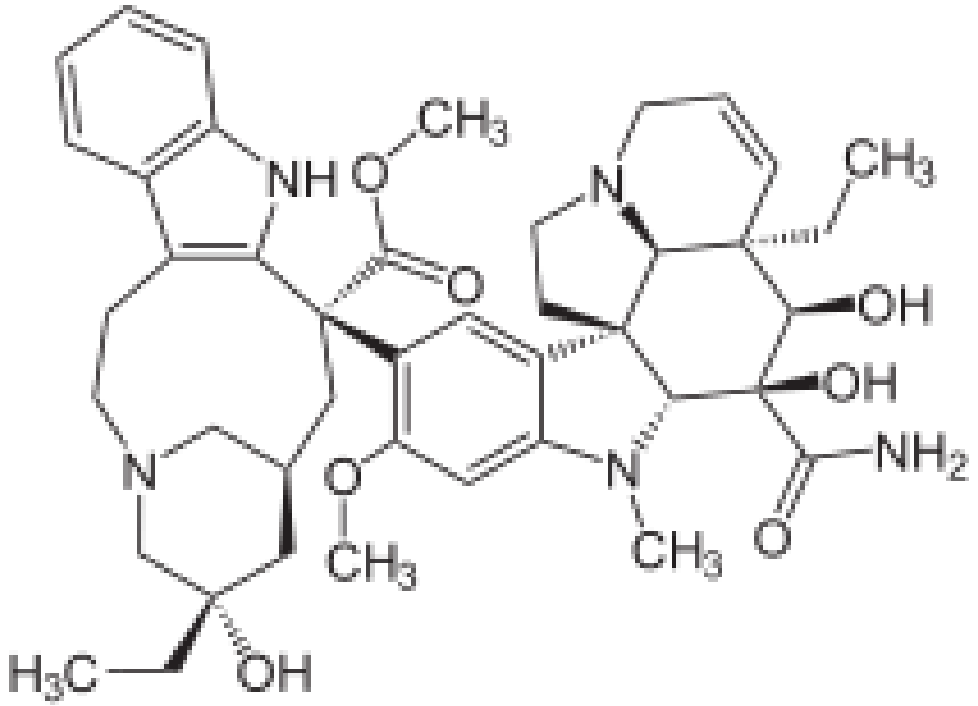
Supervised Random Walk for DILI Prediction
(DILI: Drug Induced Liver Injury)

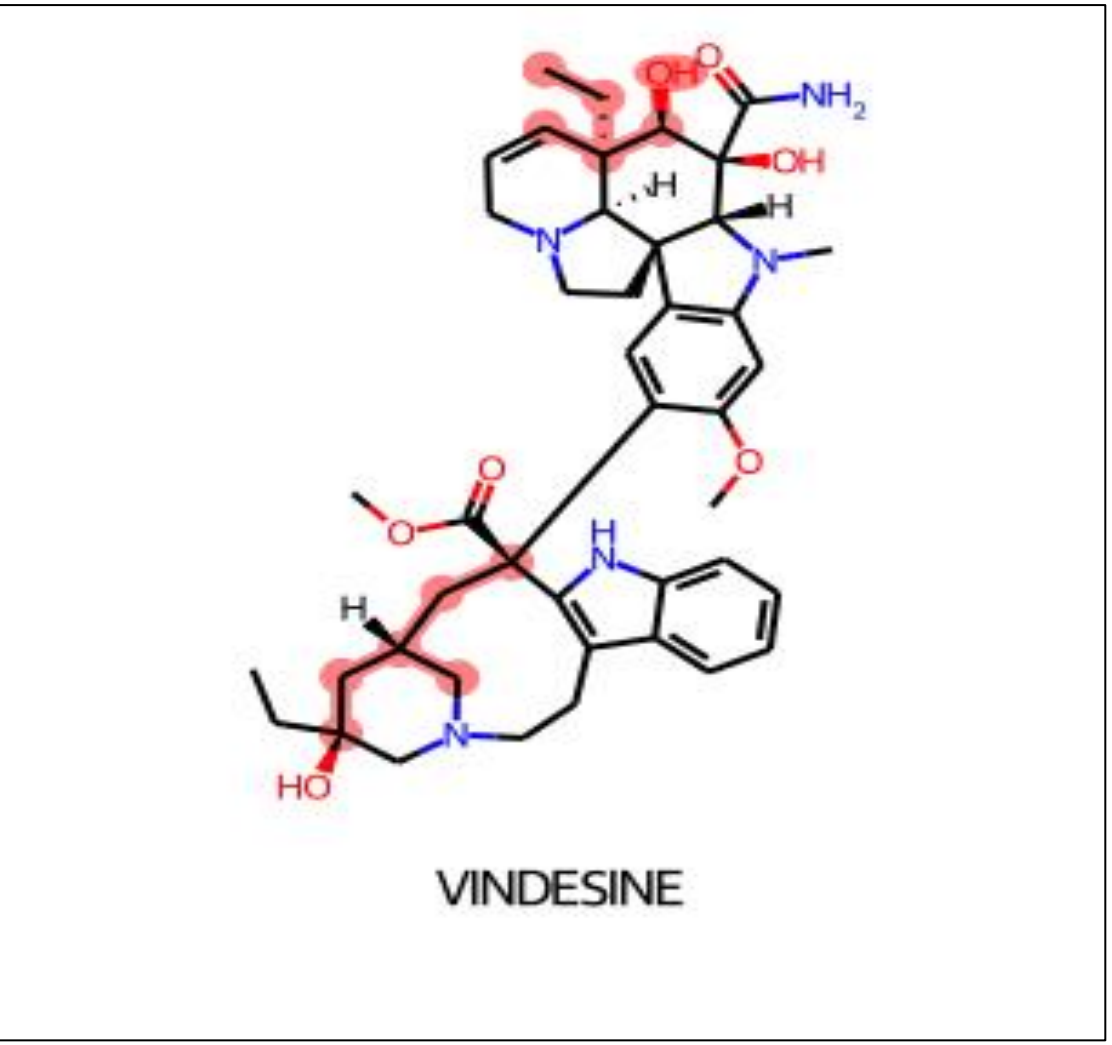
Sangsoo Lim[1], Youngkuk Kim[2], Sunho Lee[3], Jeonghyun Gu[2], Wonseok Shin[2], Sun Kim[2,3]

[1]Dongguk University
[2]Seoul National University
[3]AIGENDRUG Co., Ltd.

iScience 2023

Question: Is the compound below toxic or DILI positive?





Vindesine is an inhibitor of mitosis that is used as a chemotherapy drug

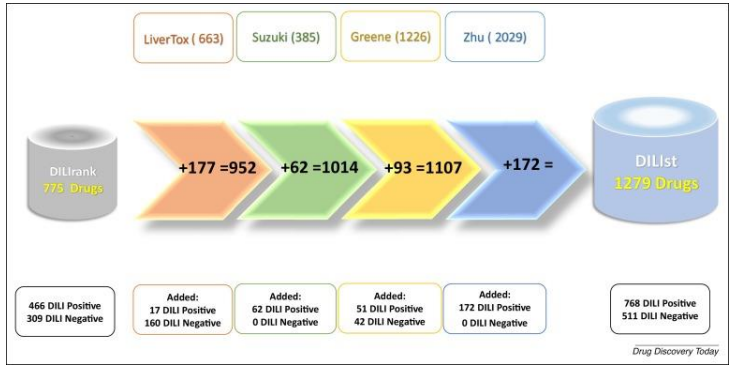
We determine vindesine as toxic drug by identifying two subgraphs of vindesine that are frequent in DILI positive drugs.

DILI Benchmark Data Sets

- **US FDA – DILIst**
  (DILIrank + LiverTox + Suzuki + Greene + Zhu)

Pre-defined Split (temporal)	Train	Valid	Test
Positive	455	-	148
Negative	293	-	98

To investigate whether the developed model could utilize the accumulated DILI information from early approved drugs to predict later approved ones, we chronologically divided the 1002 drugs into training and test sets based on the initial approval year. The year 1997 was used as a threshold since the Food and Drug Administration Modernization Act (FDAMA) (https://www.fda.gov/regulatoryinformation/selected-amendments-fdc-act/food-and-drugadministration-modernization-act-fdama-1997) was implemented at that time. The FDAMA of 1997 aimed to promote regulatory evaluation by adopting emerging technologies and eliminate unexpected adverse drug reactions in drug products. The drugs approved before and after 1997 were divided into a training set and test set, respectively. Accordingly, the training set consisted of 753 drugs (455 DILI positive/298 DILI negative), and the test set included 249 drugs (149 DILI positive/100 DILI negative).



Li, Ting, et al. *Chemical Research in Toxicology* 34.2 (2020): 550-565.

- **TDC – DILI**
  (NCTR + Greene + Xu)

Benchmark split	Train	Valid	Test
Positive	175	11	50
Negative	150	43	46

Huang, Kexin, et al. *NeurIPS* (2021).
Xu, Youjun, et al. *Journal of chemical information and modeling* 55.10 (2015): 2085-2093.

[NCTR data set]
- Chen, M.; Hong, H.; Fang, H.; Kelly, R.; Zhou, G.; Borlak, J.; Tong, W. *Toxicol. Sci.* **2013**, 136, 242.
- Chen, M.; Vijay, V.; Shi, Q.; Liu, Z.; Fang, H.; Tong, W. *Drug Discovery Today* **2011**, 16, 697−703.

[Greene data set]
- Greene, N.; Fisk, L.; Naven, R. T.; Note, R. R.; Patel, M. L.; Pelletier, D. J. *Chem. Res. Toxicol.* **2010**, 23, 1215− 1222.

[Xu data set]
- Xu, J. J.; Henstock, P. V.; Dunn, M. C.; Smith, A. R.; Chabot, J. R.; de Graaf, D. *Toxicol. Sci.* **2008**, 105, 97−105.

Our Approach

- In the human liver, drug metabolizes in a structure-dependent manner (e.g. CYP enzymes).

  → Graph based approach for subgraph identification

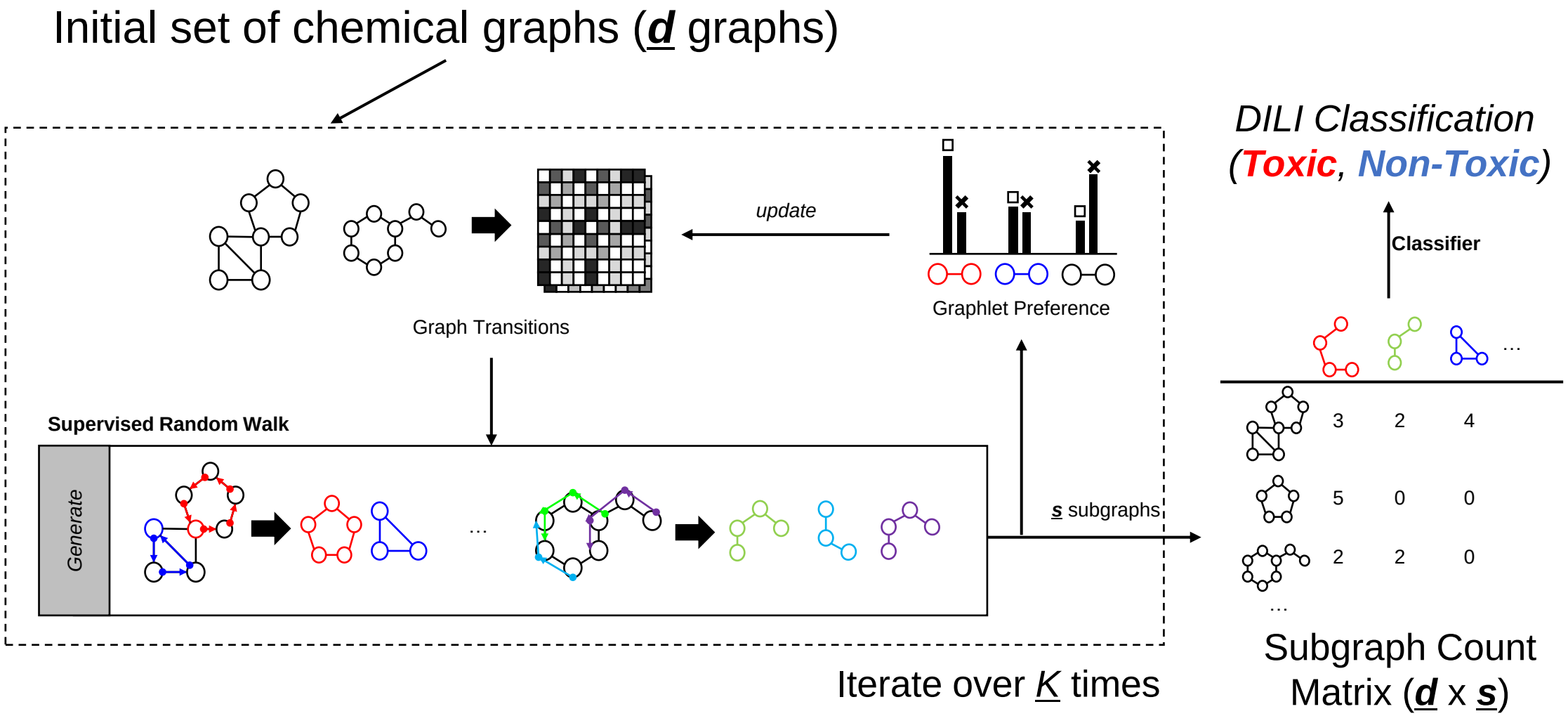
- Identifying supervised subgraph features (toxicophore) can provide understanding on the mechanism of DILI.

  → Supervised Random Walk

- There are favorable/avoidable structural patterns in drug design.

  → Frequent subgraph patterns by SMARTS

Workflow of Our Approach



Performance Comparison

a) Performance – DILIst data

Method	Description	AUC	
DeepDILI		0.659	← SOTA
GraphLOG		0.566	GNN Methods
MolHGCN		-	
Unsupervised (*ICLR*, 2020)	AttrMasking	0.672	GIN Methods
	EdgePred	0.611	
	ContextPred	0.627	
	InfoMax	0.665	
SSM (Our Approach)	Unsupervised	0.669	
	Random Forests (RF)	0.693	
	Feature Selection + RF (Features: 10,459 → 2,537)	0.694	
	Ensemble Classifier (RF + MLP)	**0.720**	

b) Performance - TDC data

Method	Description	AUC	
Source: TDC Benchmark	XGBoost	0.925	← SOTA
	AttrMasking	0.919	
	SimGCN	0.909	
	AttentiveFP	0.886	
	DeepPurpose (RDKit2D + MLP)	0.875	
	ContextPred	0.861	
	GCN	0.859	
	NeuralFP	0.851	
	DeepPurpose (Morgan + MLP)	0.832	
	DeepPurpose (CNN)	0.792	
GraphLOG		0.820	
MolHGCN		-	
SSM (Our Approach)	Unsupervised	0.929	
	Random Forests (RF)	**0.934**	
	Feature Selection + RF (Features: 10,973 → 790)	0.880	
	Ensemble Classifier (RF + MLP)	0.927	

Mining Frequent Subgraph Patterns by SMARTS

- Goal: Identifying common subgraph patterns

- Combination of ***N*** subgraphs (N = 2, 3, …)

  {subgraph$_1$}.{subgraph$_2$}…{subgraph$_N$}

- Subgraphs to SMARTS (e.g. $\underbrace{CCC=O}_{Sub_1}.\underbrace{CC(C)C=O}_{Sub_2}$) → 2-mer

- Find exactly matched *frequent* & *non-overlapping* subgraph patterns in data sets.
  - Use subgraphs of minimum support: 1% → 943 subgraphs

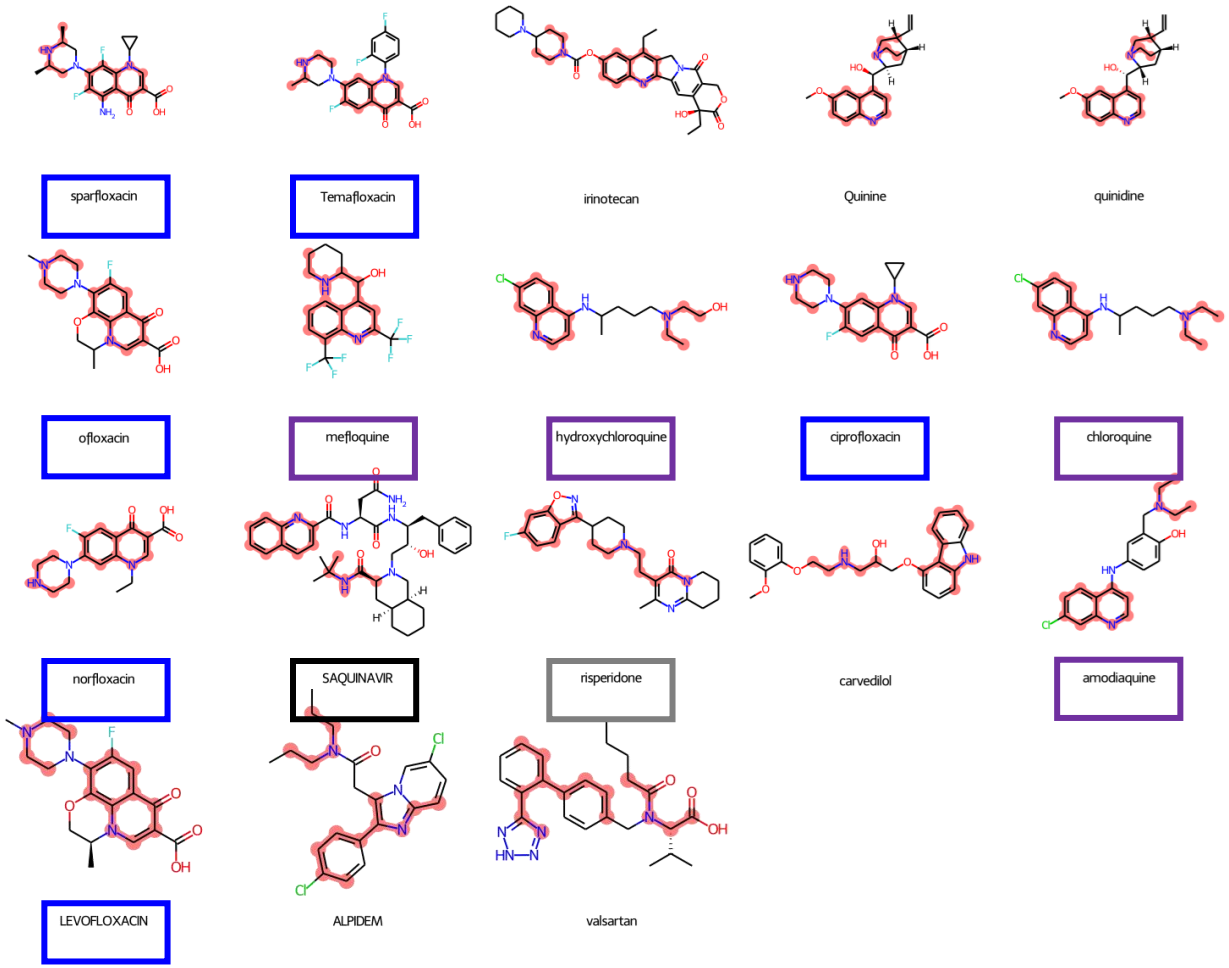
*SMILES (**S**implified **M**olecular-**I**nput **L**ine **E**ntry **S**ystem): String formatted representation of chemical compounds
*SMARTS (**SM**ILES **AR**bitrary **T**arget **S**pecification): Improved version of SMILES to specify substructural patterns in molecules.

Partial Credit: 신원석 (SNU)

Drug Class specific Frequent DILI Patterns

- In FDA: DILIst data set,

  Pattern: {ccn}.{ccccccc}.{CCNCC} only found in DILI-positive compounds.



$Support_{Tox}$: 3.96%
$Support_{NonTox}$: 0.00%

Quinolone antibiotic
Antimalarial
Carbonic anhydrase inhibitor
Penicillin antibiotic
Antiviral / anti-flu
antineoplastics
Atypical antipsychotic

Generalizing substructures with deep learning technologies

Molecular Property Prediction through Fragment-based Bi-directional Hierarchical Graph Neural Network

Dohyeon Kim

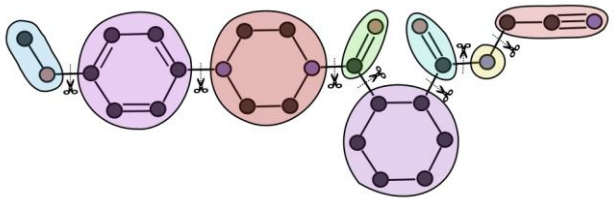
Under review

Model Architecture – Fragmentation Methods

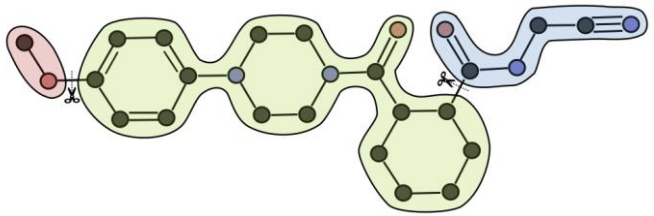
Constructed a Hierarchical Graph Structures Using Fragmentation Methods

- **Fragmentation Methods**

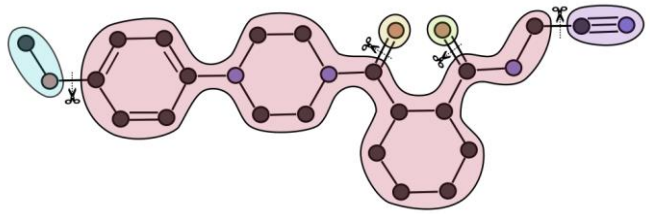
BRICS - breaks retrosynthetic bonds



Murcko - captures core scaffold structure

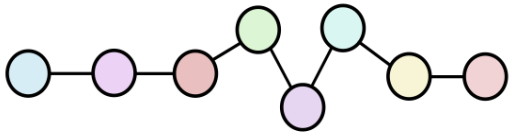


Functional Group - leaves out functional groups

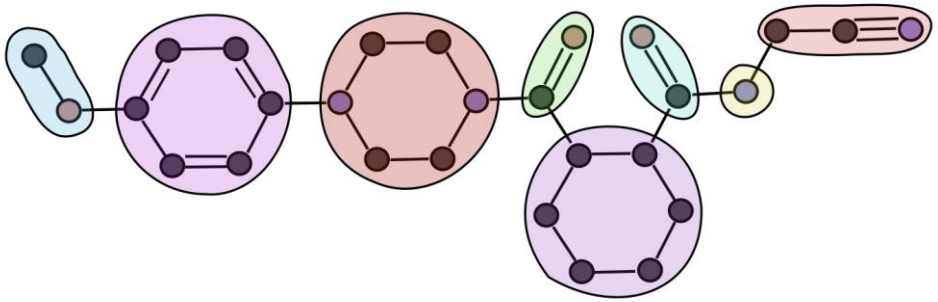


- **Hierarchical Graph Structure**

Fragment-level Graph



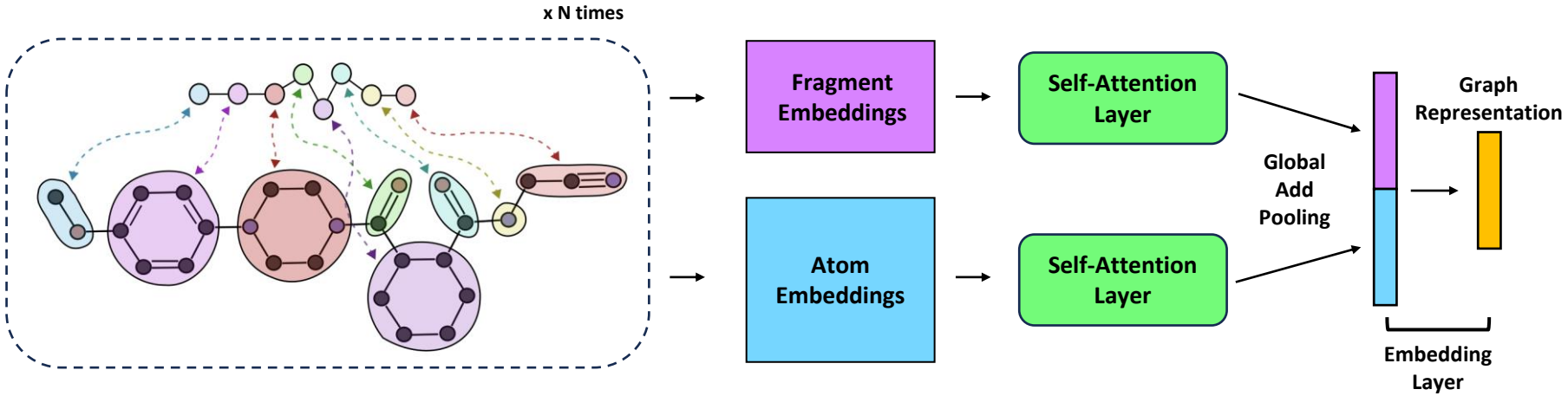
Atom-level whole Graph



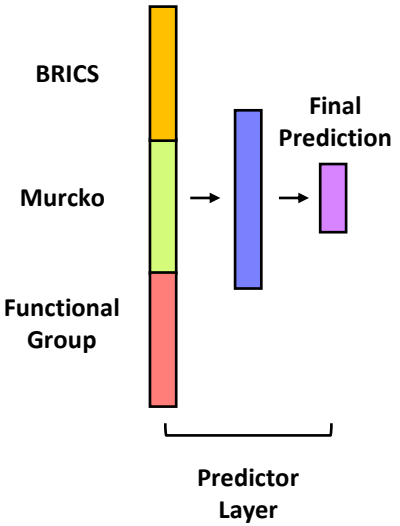
GIN message passing algorithm
for both atom and fragment-level graph

Model Architecture - Overview

Graph Representations from Different Fragmentation Methods



Concatenate Graph Representations for Final Prediction



Model Architecture – Self-Attention

Self-Attention to Improve Interpretability

G transformed into $Q_i,\ K_i,\ V_i$

$$G = \{h_1^{(k)}, ..., h_v^{(k)}, ..., h_n^{(k)}\} \in \mathbb{R}^{n \times d_{\text{model}}}$$

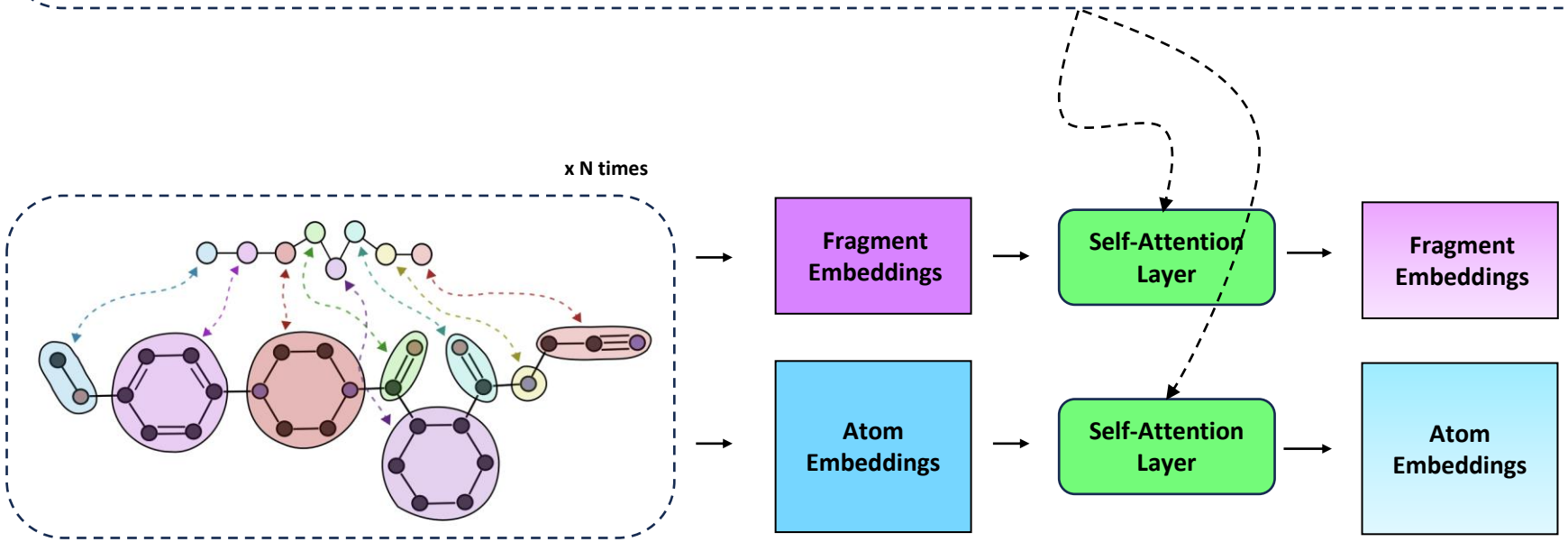
$$Q_i = GW_i^Q,\ K_i = GW_i^K,\ V_i = GW_i^V$$

Multi-Head Attention

$$\text{Attention}\,(Q_i, K_i, V_i) = \text{softmax}(\frac{Q_i K_i^T}{\sqrt{d_k}})V_i$$

$$\text{MultiHead}\,(Q, K, V) = \text{Concat}\,(\text{head}_1, ..., \text{head}_h)\,W^O$$

$$\text{where head}_i = \text{Attention}(Q_i, K_i, V_i)$$



Classification (higher is better ↑) ROC-AUC %					
Datasets	BBBP	Clintox	Tox21	ToxCast	SIDER
# Molecules	2039	1478	7831	8575	1427
# Tasks	1	2	12	617	27
D-MPNN	71.0(0.3)	**90.5(0.6)**	75.9(0.7)	65.5(0.3)	57.0(0.7)
AttentiveFP	64.3(1.8)	84.7(0.3)	76.1(0.5)	63.7(0.2)	60.6(3.2)
Frag-BHGNN	**76.3(1.4)**	80.3(3.6)	**80.8(0.2)**	**74.9(1.0)**	**64.6(1.4)**

Table 1: Frag-BHGNN performance on molecular property prediction classification tasks

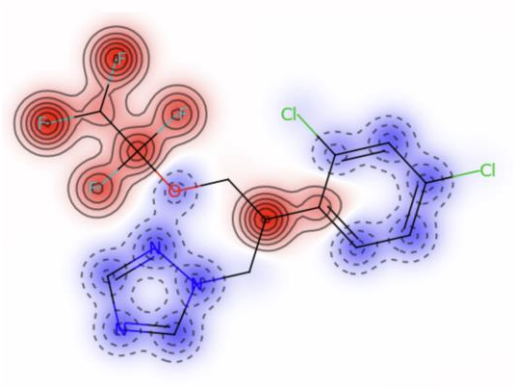
Attention Visualization - ToxCast

TOX21_Aromatase_Inhibition : inhibition of the enzyme **aromatase**

- Aromatase : an enzyme that plays a critical role in the biosynthesis of estrogens.

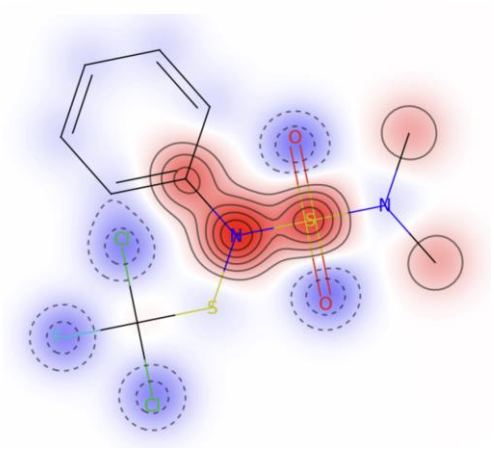
TETRACONAZOLE

TOX21_Aromatase_Inhibition : 1.0
Prediction : 0.76



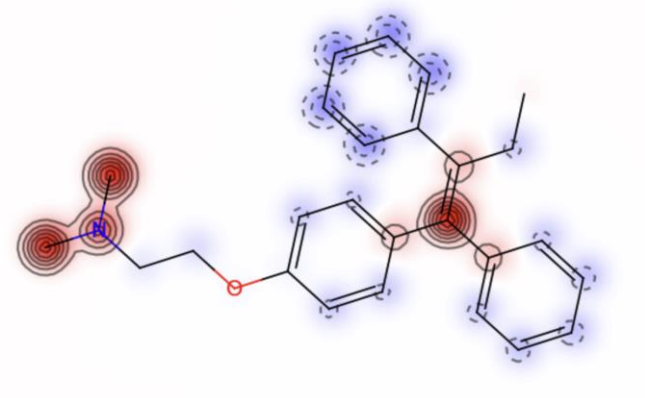
DICHLOFLUANID

TOX21_Aromatase_Inhibition : 1.0
Prediction : 0.81



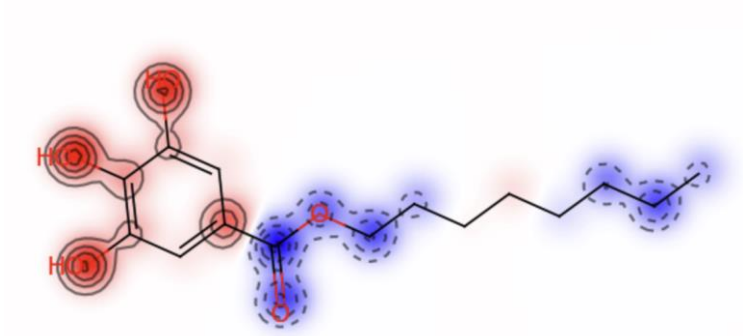
TAMOXIFEN CITRATE

TOX21_Aromatase_Inhibition : 1.0
Prediction : 0.89



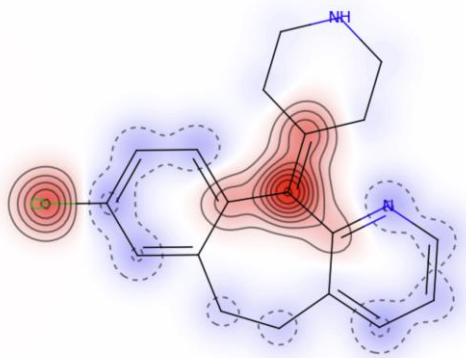
OCTYL_GALLATE

TOX21_Aromatase_Inhibition : 1.0
Prediction : 0.89



DESLORATADINE

TOX21_Aromatase_Inhibition : 1.0
Prediction : 0.88



Modeling bioassays simultaneously for modeling toxicity

Multi-Task Aware Learnable Prototypes on Few Shot learning for Molecular Property Prediction

Dr. Sangseon Lee

Under review

Why Multi-task Learning?
A Number of Assasy for Testing Toxicity

This study used curated data sets, **MoleculeNet** (*Chemical Science* 2019) which is standard test data sets for AI research (a little bit outdated).

- **Tox21**:
  - Qualitative toxicity measurements for 8,014 molecules with 12 tasks
- **SIDER**:
  - Drug side-effects on 27 organs according to MedDRA classification for 1,427 molecules
- **Toxcast**:
  - Qualitative toxicity measurements for 8,615 molecules with 617 tasks

Motivation of Learning Prototypes

- Molecular property prediction in few-shot learning

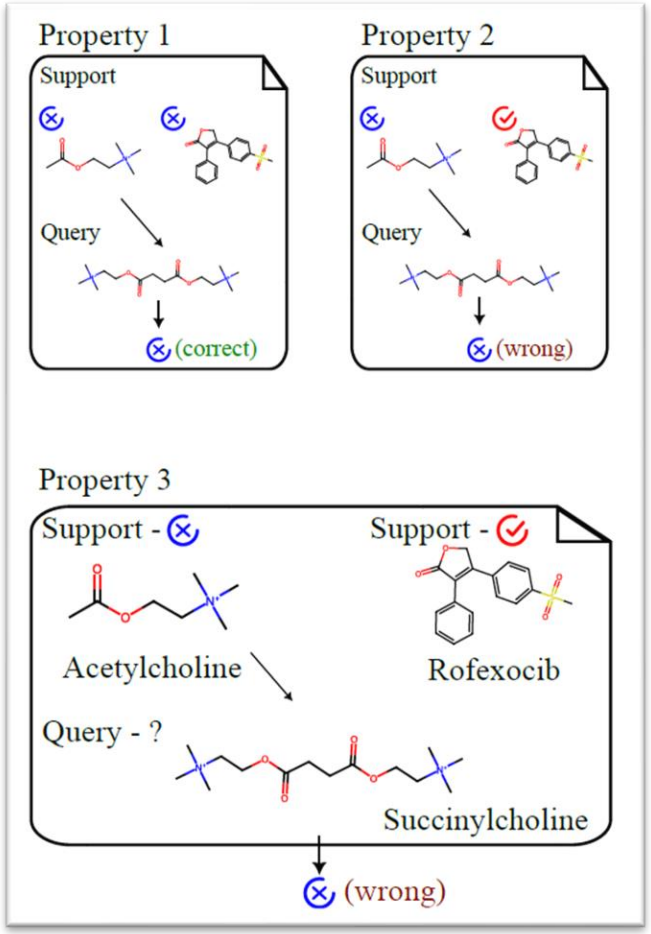
- Limitation of existing methods
  - Focus on a single property
  - Neglect interrelated properties of molecules

- Proposed approach

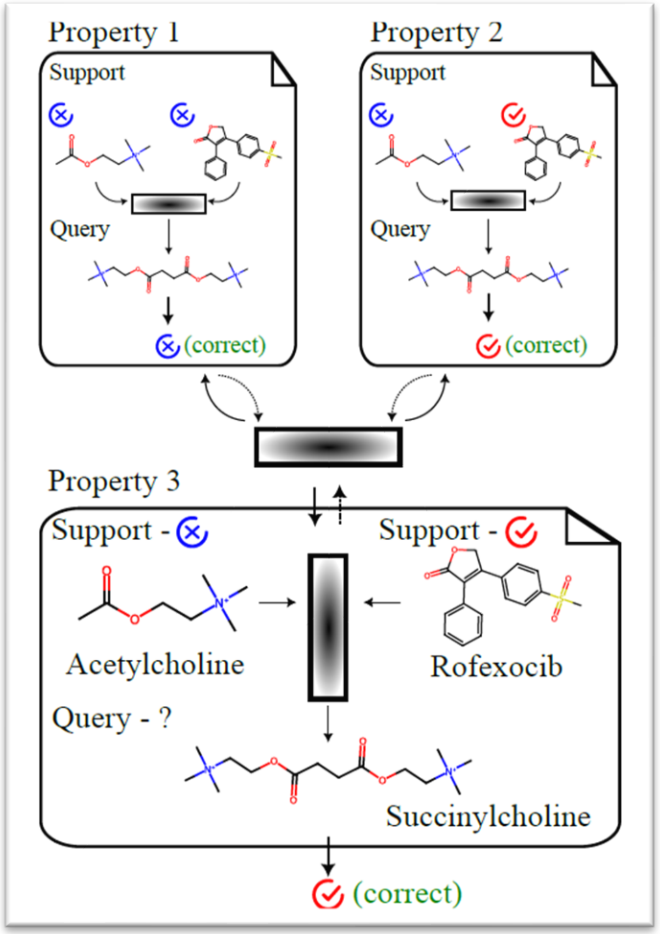
  - Learnable Prototype vectors –

  - Capture the shared knowledge among multiple interrelated molecular properties

Existing methods



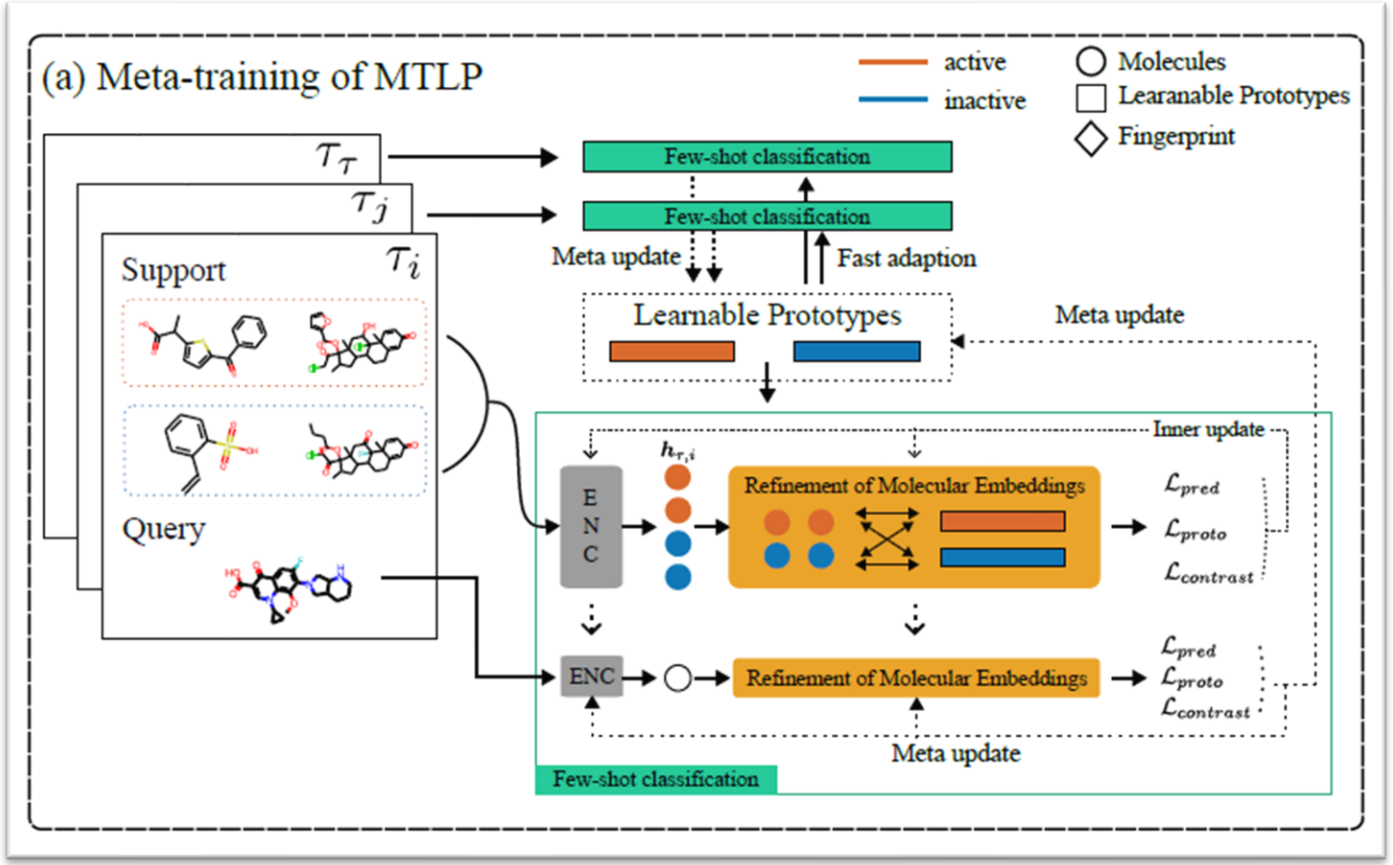
Proposed method

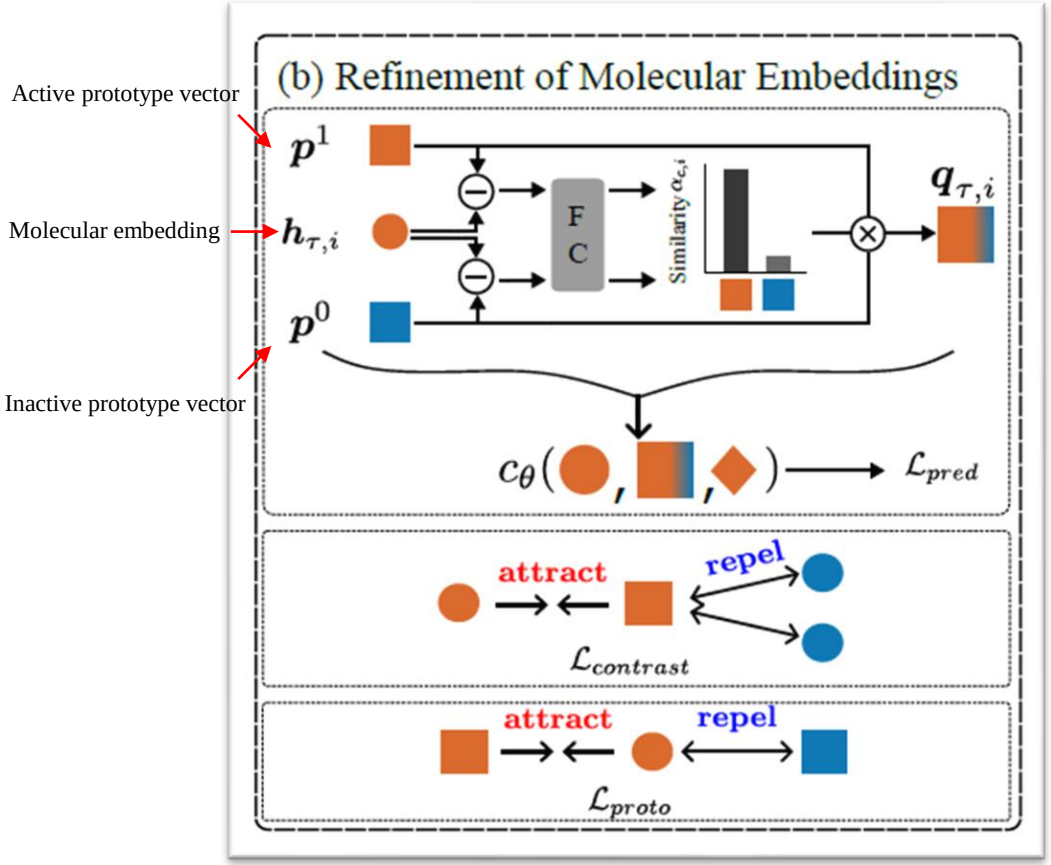


Overview of MTLP

- Multi-Task informed Learnable Prototype -

- (a) Learning of the prototype vectors in a meta-learning framework
- (b) A stochastic attention mechanism & multi-view contrastive learning losses





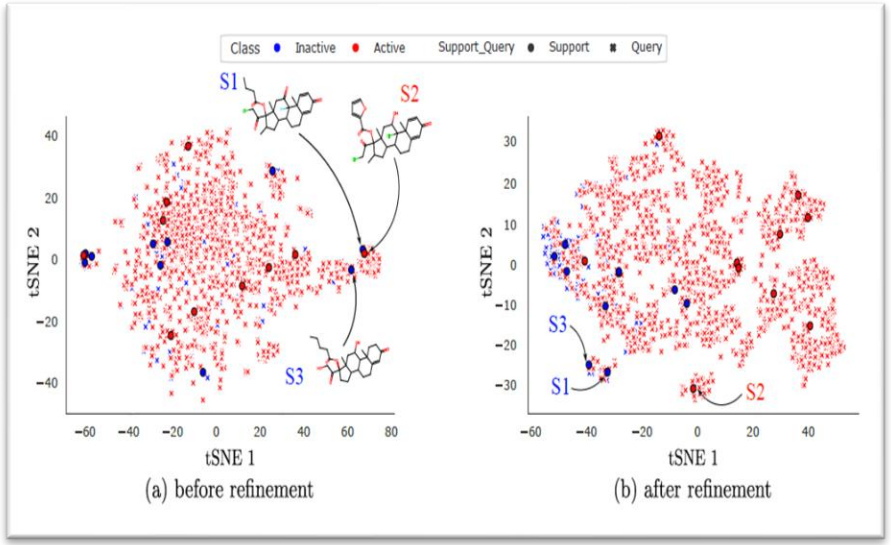
Comparison with baselines

(a) Performance on benchmark datasets

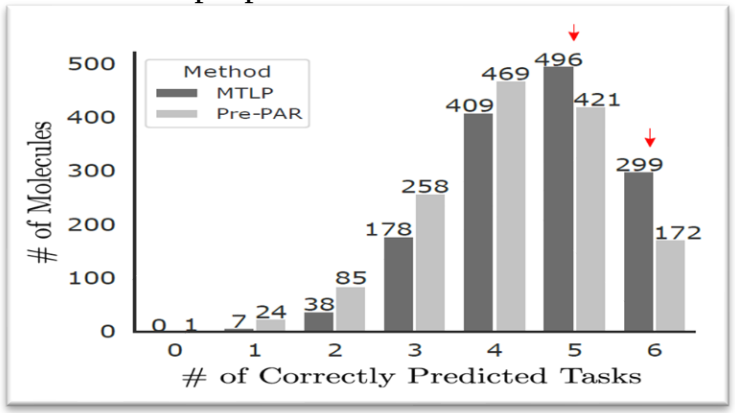
Table 1: Performance comparison on benchmark datasets for molecular property prediction. Average ROC-AUC scores with standard deviations for test tasks are reported. OOM: Out-of-Memory error, *: method utilizing pretrained graph encoder. The best results for each dataset are shown in bold.

Method	Tox21		SIDER		MUV		ToxCast	
	10-shot	1-shot	10-shot	1-shot	10-shot	1-shot	10-shot	1-shot
Siamese	$80.40_{\pm 0.35}$	$65.00_{\pm 1.58}$	$71.10_{\pm 4.32}$	$51.43_{\pm 3.31}$	$59.96_{\pm 5.13}$	$50.00_{\pm 0.17}$	-	-
ProtoNet	$74.98_{\pm 0.32}$	$65.58_{\pm 1.72}$	$64.54_{\pm 0.89}$	$57.50_{\pm 2.34}$	$65.88_{\pm 4.11}$	$58.31_{\pm 3.18}$	$63.70_{\pm 1.26}$	$56.36_{\pm 1.54}$
MAML	$80.21_{\pm 0.24}$	$75.74_{\pm 0.48}$	$70.43_{\pm 0.76}$	$67.81_{\pm 1.12}$	$63.90_{\pm 2.28}$	$60.51_{\pm 3.12}$	$66.79_{\pm 0.85}$	$65.97_{\pm 5.04}$
TPN	$76.05_{\pm 0.24}$	$60.16_{\pm 1.18}$	$67.84_{\pm 0.95}$	$62.90_{\pm 1.38}$	$65.22_{\pm 5.82}$	$50.00_{\pm 0.51}$	$62.74_{\pm 1.45}$	$50.01_{\pm 0.05}$
EGNN	$81.21_{\pm 0.16}$	$79.44_{\pm 0.22}$	$72.87_{\pm 0.73}$	$70.79_{\pm 0.95}$	$65.20_{\pm 2.08}$	$62.18_{\pm 1.76}$	$63.65_{\pm 1.57}$	$61.02_{\pm 1.94}$
IterRefLSTM	$81.10_{\pm 0.17}$	$80.97_{\pm 0.10}$	$69.63_{\pm 0.31}$	$71.73_{\pm 0.14}$	$49.56_{\pm 5.12}$	$48.54_{\pm 3.12}$	-	-
PAR	$82.06_{\pm 0.12}$	$80.46_{\pm 0.13}$	$74.68_{\pm 0.31}$	$71.87_{\pm 0.48}$	$66.48_{\pm 2.12}$	$64.12_{\pm 1.18}$	$69.72_{\pm 1.63}$	$67.28_{\pm 2.90}$
Pre-GNN*	$82.14_{\pm 0.08}$	$81.68_{\pm 0.09}$	$73.96_{\pm 0.08}$	$73.24_{\pm 0.12}$	$67.14_{\pm 1.58}$	$64.51_{\pm 1.45}$	$73.68_{\pm 0.74}$	$72.90_{\pm 0.84}$
Pre-PAR*	$84.93_{\pm 0.11}$	$83.01_{\pm 0.09}$	$78.08_{\pm 0.16}$	$74.46_{\pm 0.29}$	$69.96_{\pm 1.37}$	$66.94_{\pm 1.12}$	$75.12_{\pm 0.84}$	$73.63_{\pm 1.00}$
Meta-MGNN*	$82.97_{\pm 0.10}$	$82.13_{\pm 0.13}$	$75.43_{\pm 0.21}$	$73.36_{\pm 0.32}$	$68.99_{\pm 1.84}$	$65.54_{\pm 2.13}$	OOM	OOM
MTLP* (ours)	$\mathbf{86.04}_{\pm 0.15}$	$\mathbf{83.55}_{\pm 0.15}$	$\mathbf{84.36}_{\pm 0.11}$	$\mathbf{81.19}_{\pm 0.31}$	$\mathbf{72.74}_{\pm 0.85}$	$\mathbf{69.09}_{\pm 1.26}$	$\mathbf{76.03}_{\pm 0.09}$	$\mathbf{75.13}_{\pm 0.05}$

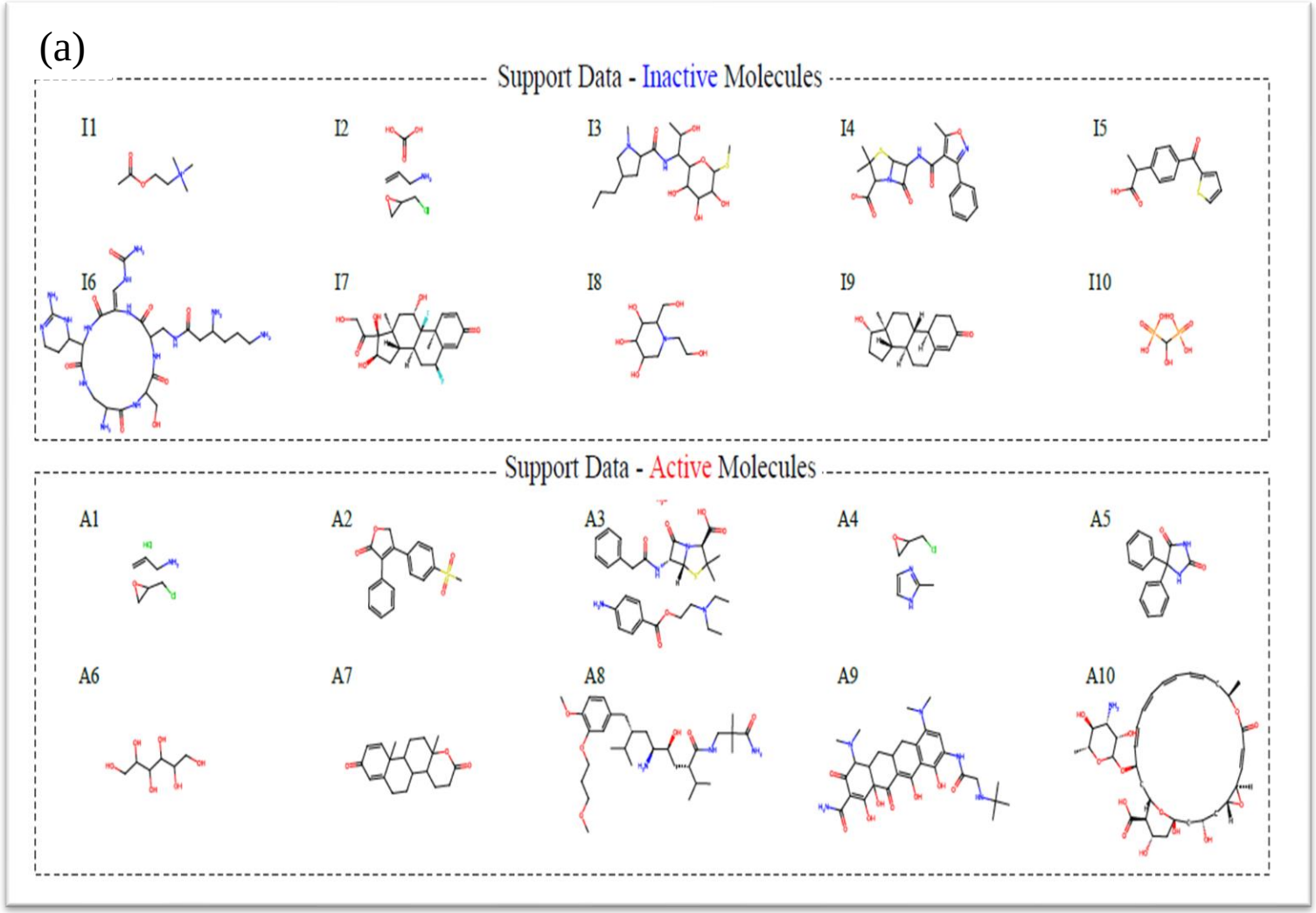
(b) Visualization of a chemical space

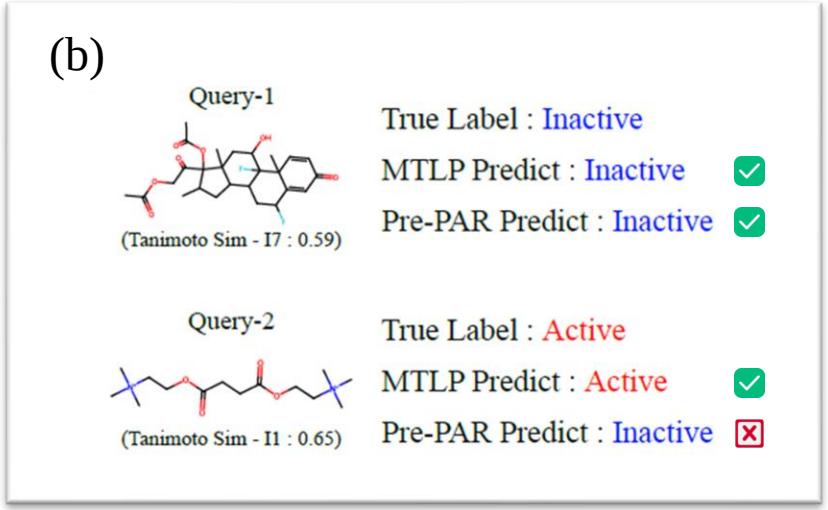


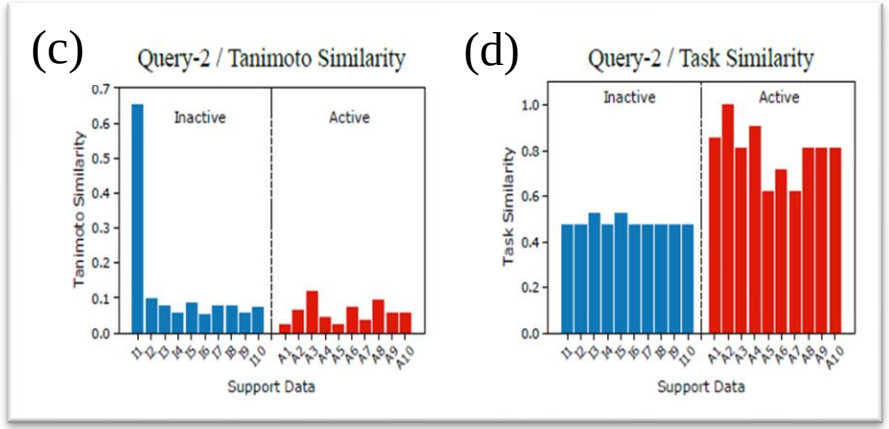
(c) Ratio of accurately predicting multiple molecular properties.



Examples of Few-shot Prediction







Predicting side effects with deep learning technologies

Dual representation learning for predicting drug-side effect frequency using protein target information

Sungjoon Park†, Sangseon Lee†, Minwoo Pak, and Sun Kim*

IEEE Journal of Biomedical and Health Informatics 2024

Dataset

- 750 drugs × 994 side effects from SIDER database
  - frequency ∈ {0, 1, 2, 3, 4, 5}

- Drug features

  : molecular graph, drug targets, drug-drug similarity

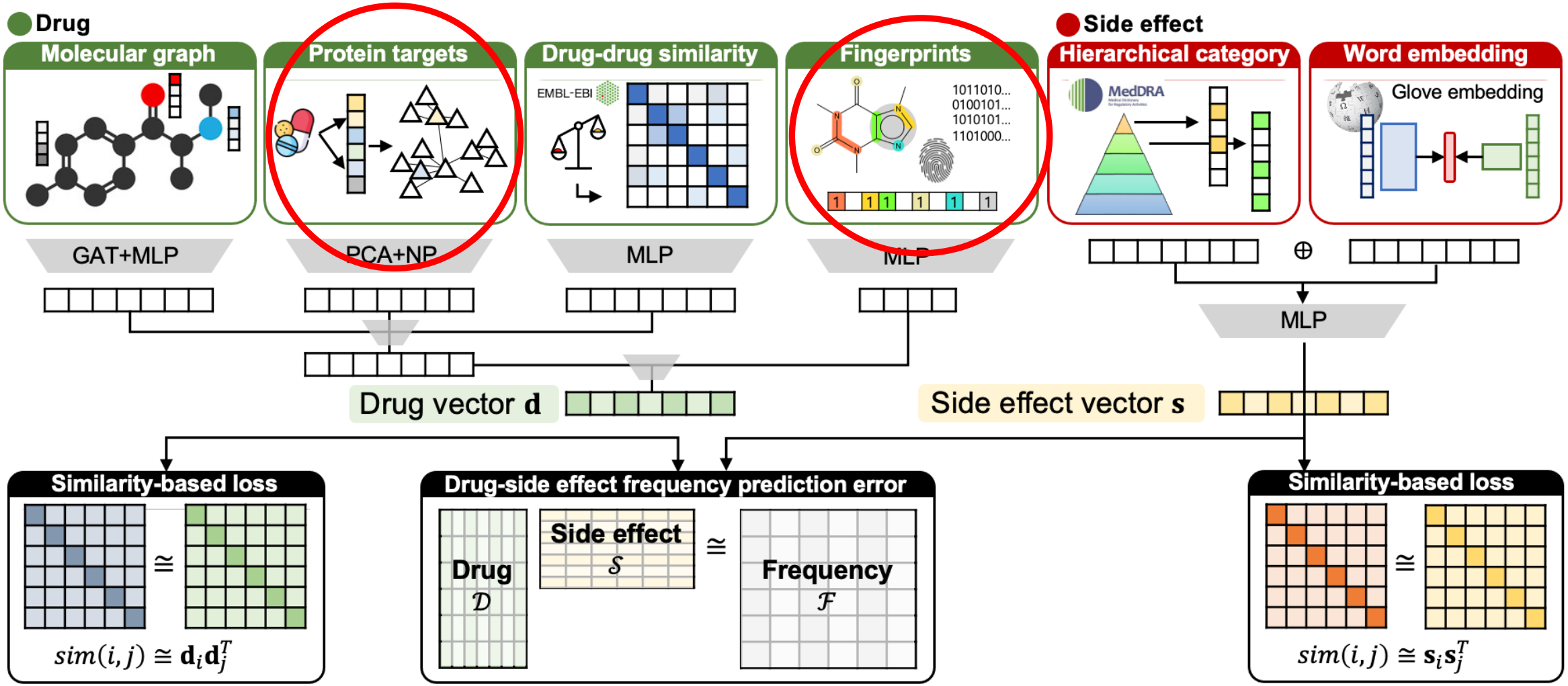
  SMILES string DrugBank STITCH

- Side effect features

  : MedDRA category, GloVe word embedding

  MedDRA Wikipedia

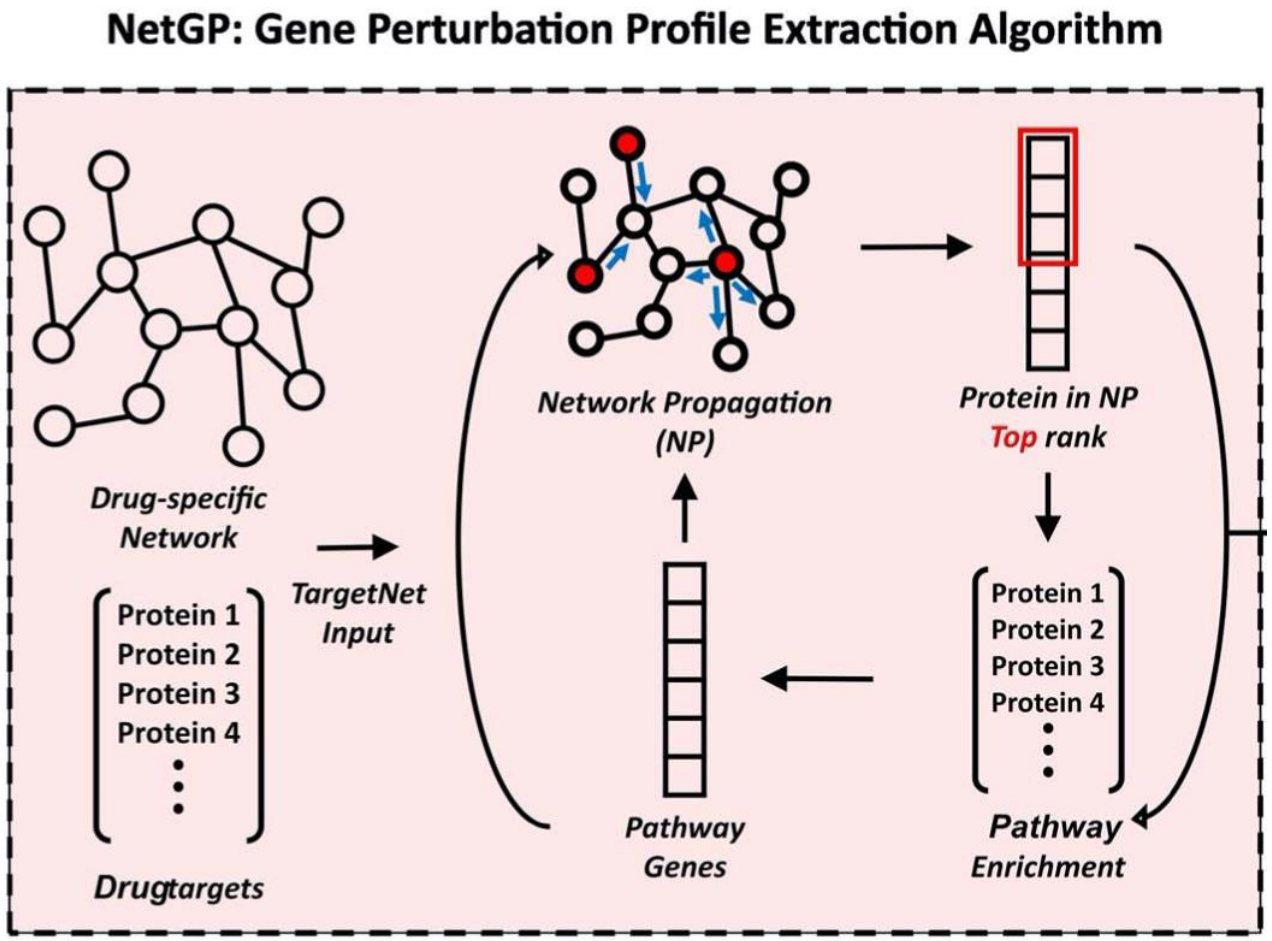
Model architecture



Drug protein target encoding

1.
Construct drug-specific protein-protein interaction (PPI) network

2.
Start network propagation (NP) with target proteins as seed genes



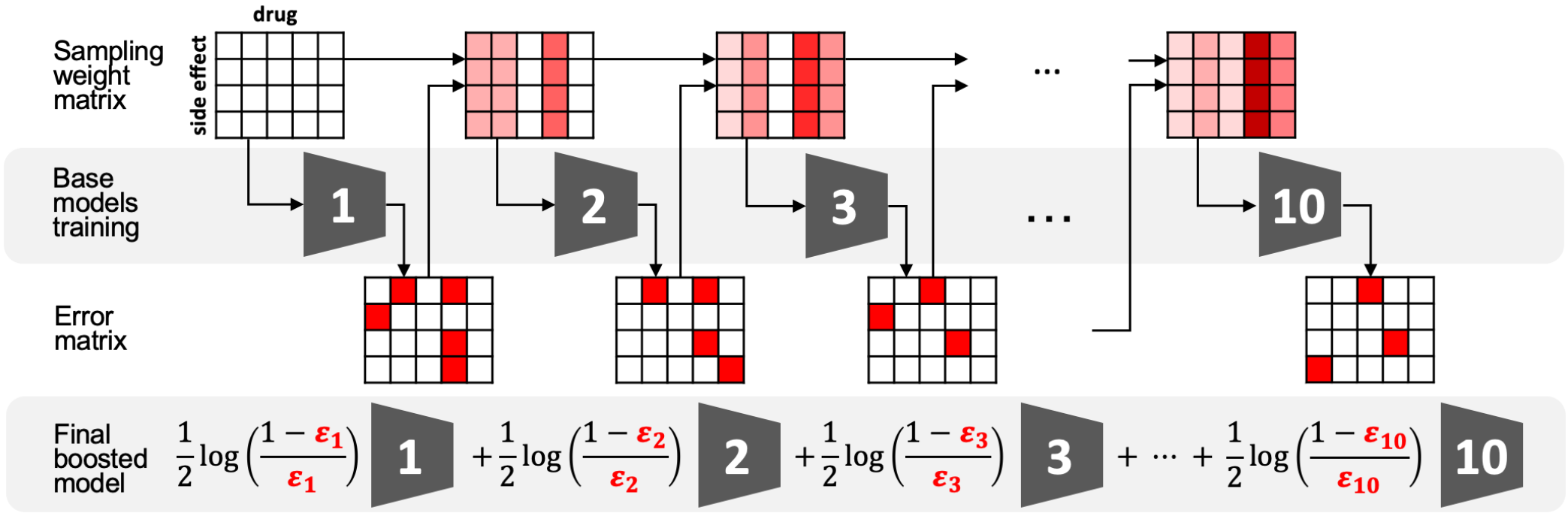
3.
Get top 100 proteins as a result of NP

4.
Get genes of enriched pathway as new seed genes;
repeat until convergence

Pak, Minwoo, et al. "Improved drug response prediction by drug target data integration via network-based profiling." Briefings in Bioinformatics 24.2 (2023): bbad034.

Adaboost

- An ensemble method to rebalance the sampling weights for the training data
- Effectively integrates the use of heterogeneous drug features



Results

Metric	MGPred [28]	SDPred [30]	DSGAT [31]	Our mode
SCC	-0.065	0.258	0.431	**0.438**
RMSE	3.435	3.649	1.470	**1.407**
MAE	3.314	3.539	1.174	**1.057**
AUROC	0.746	0.845	0.878	**0.901**
mAP (AUPRC)	0.178	0.347	0.403	**0.436**
nDCG@10	0.201	0.778	0.813	**0.858**
Precision@1	0.019	0.668	0.701	**0.750**
Precision@15	0.021	0.476	0.513	**0.556**
Recall@1	0.000	0.026	0.030	**0.031**
Recall@15	0.004	0.241	0.265	**0.267**

[28] *Briefings in Bioinformatics*, vol. 22, no. 6, 2021.
[30] *Briefings in Bioinformatics*, vol. 23, no. 1, 2022.
[31] *Briefings in Bioinformatics*, vol. 23, no. 2, 2022.

Results (cont'd)

- External validation using independent nine drugs

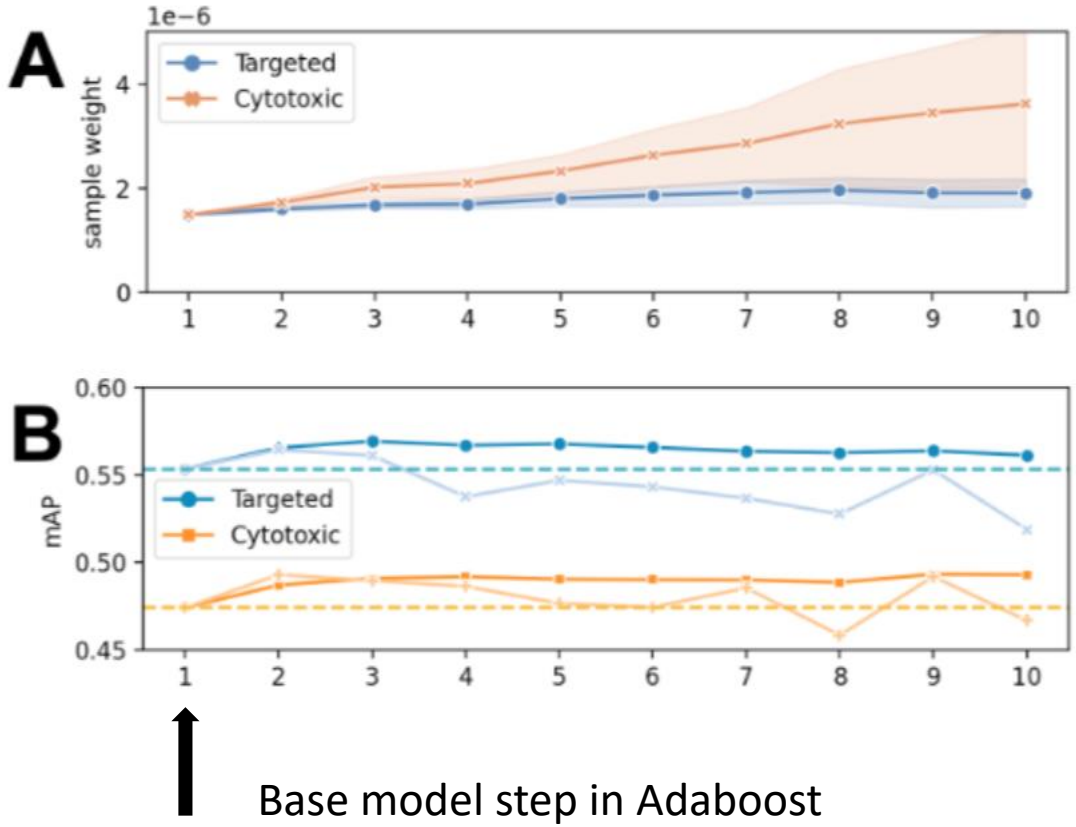
- Fully utilizes drug-drug similarity features

Table 3. Independent nine drugs performance. We trained our model on all 750 drugs and 994 side effects, and tested on nine novel drugs. The prediction of drug-side effect frequency for these drugs do not deteriorate with external data.

Drug name	SCC	RMSE	MAE	AUROC	mAP
balsalazide	0.351	1.067	0.693	0.953	0.712
carboplatin	0.204	0.976	0.786	0.952	0.478
cisatracurium	-*	0.517	0.448	0.971	0.292
doxercalciferol	0.327	1.404	1.166	0.955	0.420
esomeprazole	-0.144	1.678	1.317	0.937	0.554
everolimus	0.500	1.676	1.370	0.871	0.682
fidaxomicin	0.577	0.769	0.536	0.976	0.486
gadoteridol	0.434	1.059	1.796	0.898	0.470
ixabepilone	0.391	1.110	0.858	0.958	0.740
Avg.	0.330	1.140	0.997	0.941	0.537

* Only one-class label to predict

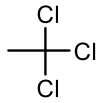
Results (cont'd)



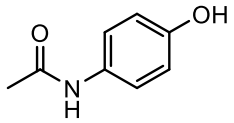
- Drugs with ambiguous target does not benefit from protein target information
- Targeted = w/ explicit targets
- Cytotoxic = w/ ambiguous targets
- Cytotoxic drugs initially show worse prediction, but improve as Adaboost continues

Ultimately, we want to show

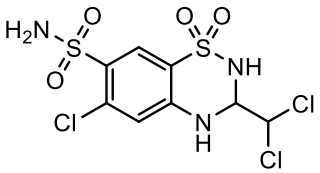
Graph Learning for Toxicity and Side Effects



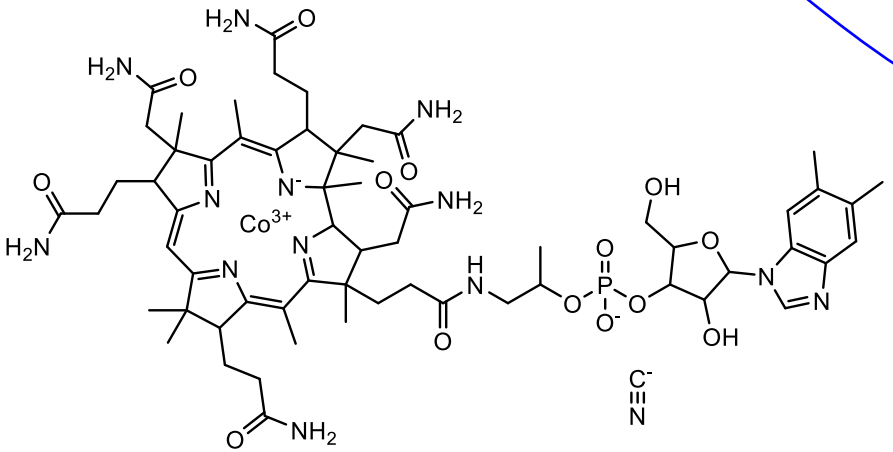
Methylchloroform
(photoresist solvent)
(central nervous system depressant)



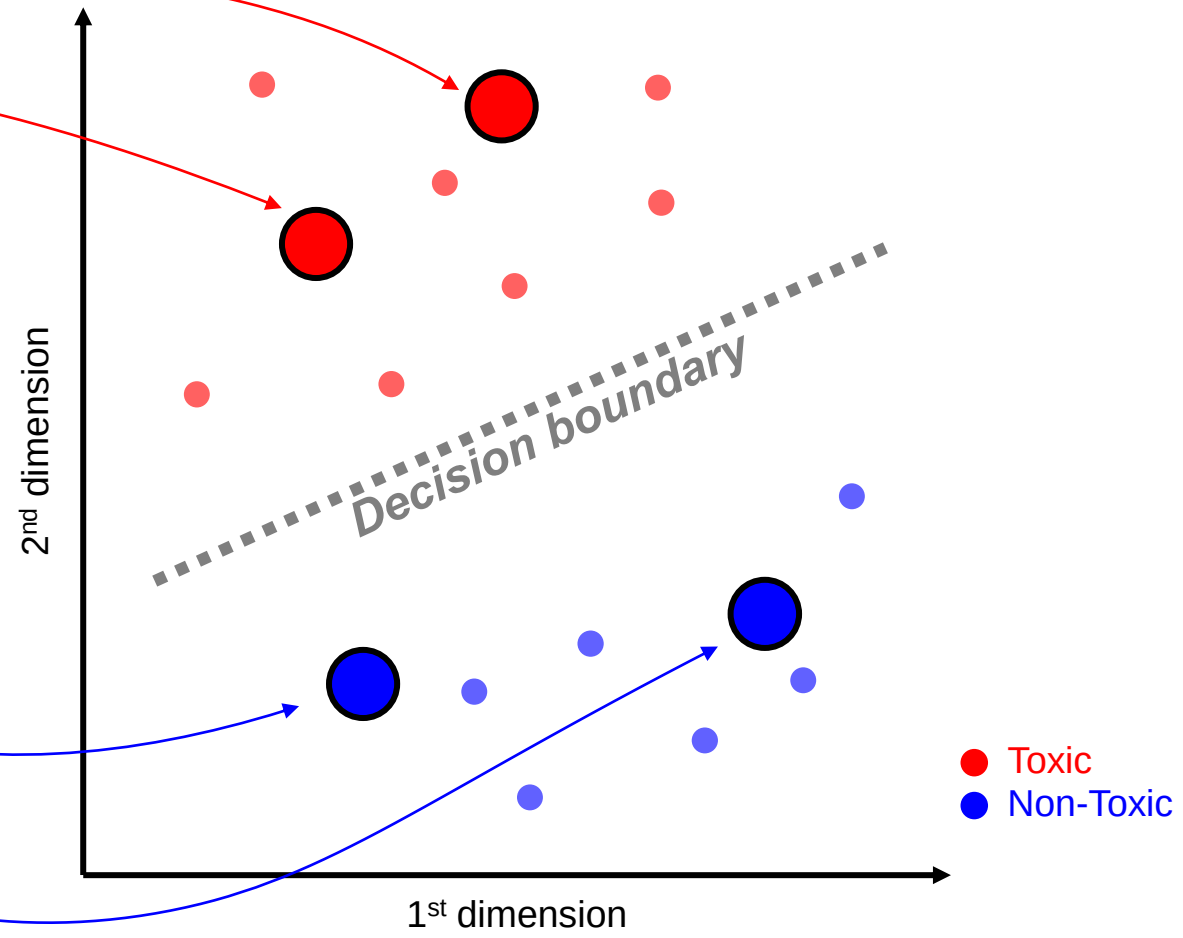
Acetaminophen
(fever/pain treatment)



Trichlormethiazide
(diuretic)



Cyanocobalamin
(vitamin B12 dificiency)



Degree of Toxicity
in terms of Genetic Space

A Multi-dimensional Transcriptomic Ruler for Liver Toxicity

Inyoung Sung[†], Sangseon Lee[†], Dongmin Bang, Jungseob Yi, and Sun Kim

Under review

Dataset

- 1,554 drug hepatotoxicity labeled data from DILIrank and LiverTox
  - Toxic labeled drugs: 456
  - Non-toxic labeled drugs: 1,098

- 17,738 drug-treated gene expression data from LINCS
  - DMSO-treated samples: 2,791
  - Non-toxic drug-treated samples: 11,333
  - Toxic drug-treated samples: 6,405

Scientific Question and Our Approach

- Which of transcriptome profile represents DILI?

- Toxicity cannnot hardly be defiend as a binary decision, toxic *vs*. non-toxic.
  - Degree of toxicty needs to be defined according to dose and treatment time.

- We construct a transcriptomic ruler for measuring degree of toxicity!

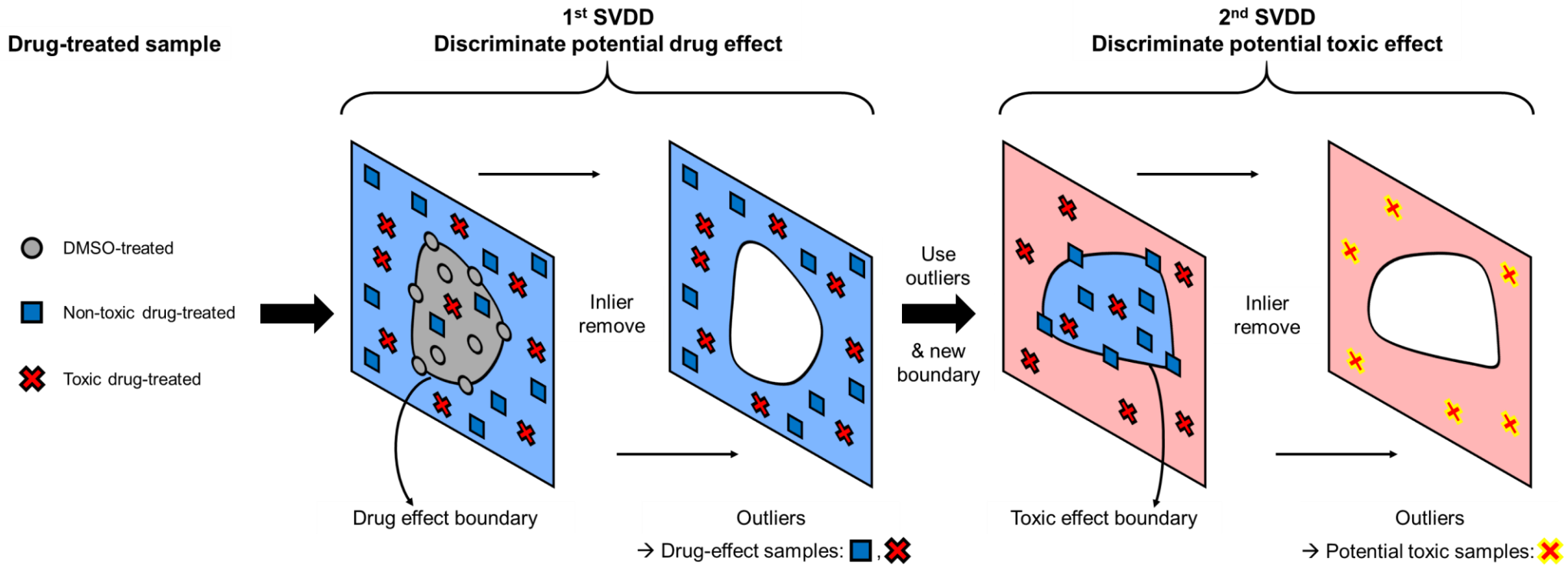
*PT: potentially toxic

Step 1: Generate dual-boundaries

- **Goal**: identification of region containing potentially toxic (PT) samples.

  Maximumly perturbed transcriptomic state → Toxic transcriptomic signature!

- Use **Dual-SVDD** to generate dual-boundaries in two steps.

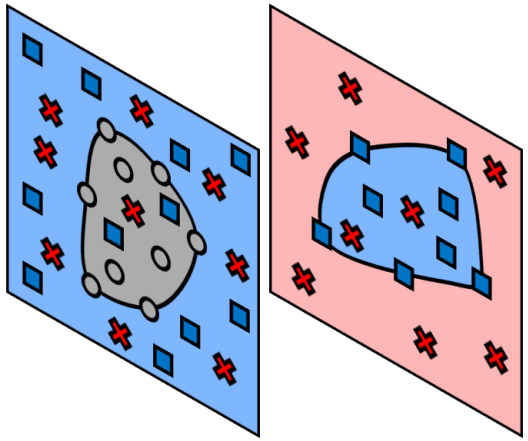


*PT: potentially toxic

Step 1: Dual-boundaries of Toxic Signature

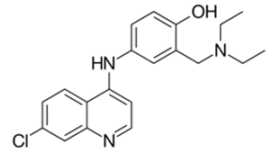
a. Dual-SVDD results

- Potential toxic (PT) samples
  : 64 drug-treated samples

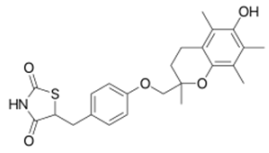


	1st boundary		2nd boundary	
	In	Out	In	Out
DMSO	2,791	0	0	0
Non-toxic	9,901	1,432	1,432	0
Toxic	5,941	464	400	64

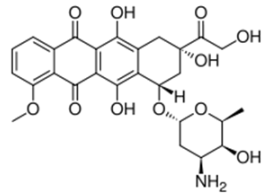
b. Examples of PT samples



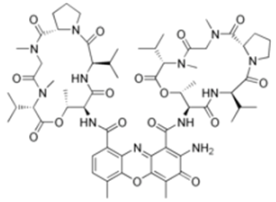
Drug	Amodiaquine
Cell line	NEU
Time	24hour
Dose	10uM



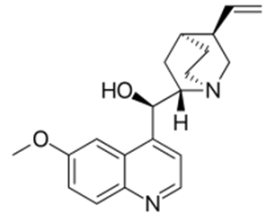
Drug	Troglitazone
Cell line	MCF7
Time	24hour
Dose	3.3uM



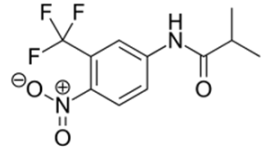
Drug	Doxorubicin
Cell line	HCC515
Time	24hour
Dose	10uM



Drug	Dactinomycin
Cell line	VCAP
Time	24hour
Dose	10uM



Drug	Quinine
Cell line	SW948
Time	6hour
Dose	10uM



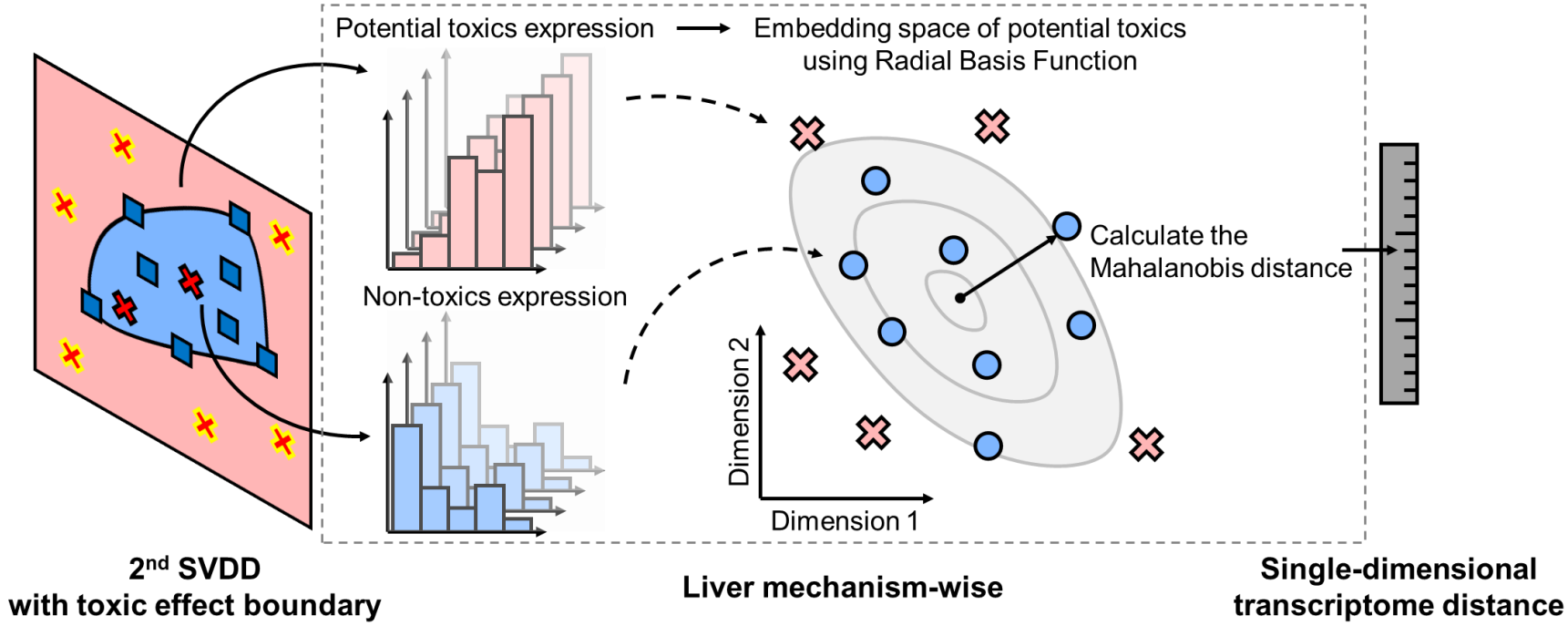
Drug	Flutamide
Cell line	SNUC5
Time	6hour
Dose	30uM

*RBF: Radial Basis Function

Step 2: Define of a liver toxicity distance

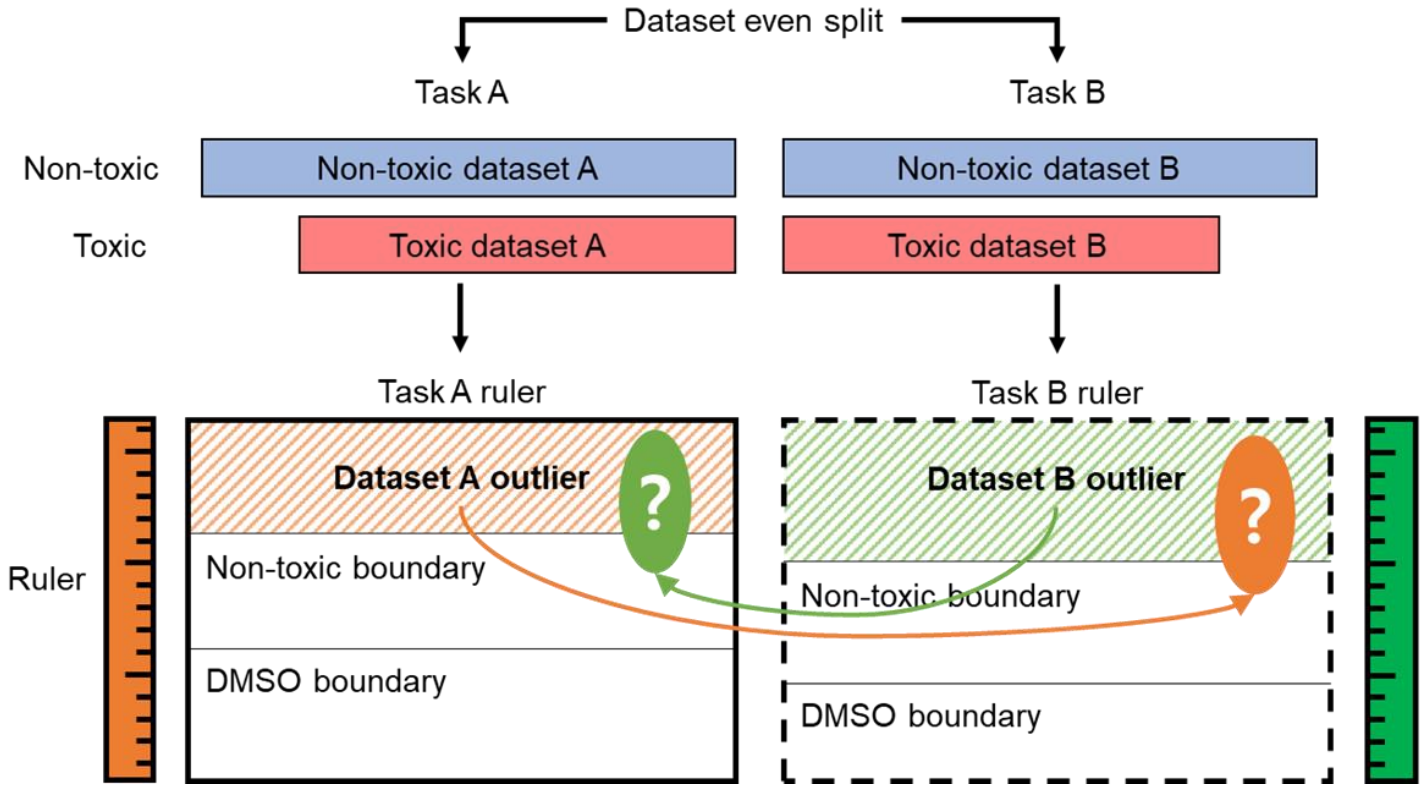
*Dysregulation of mechanism

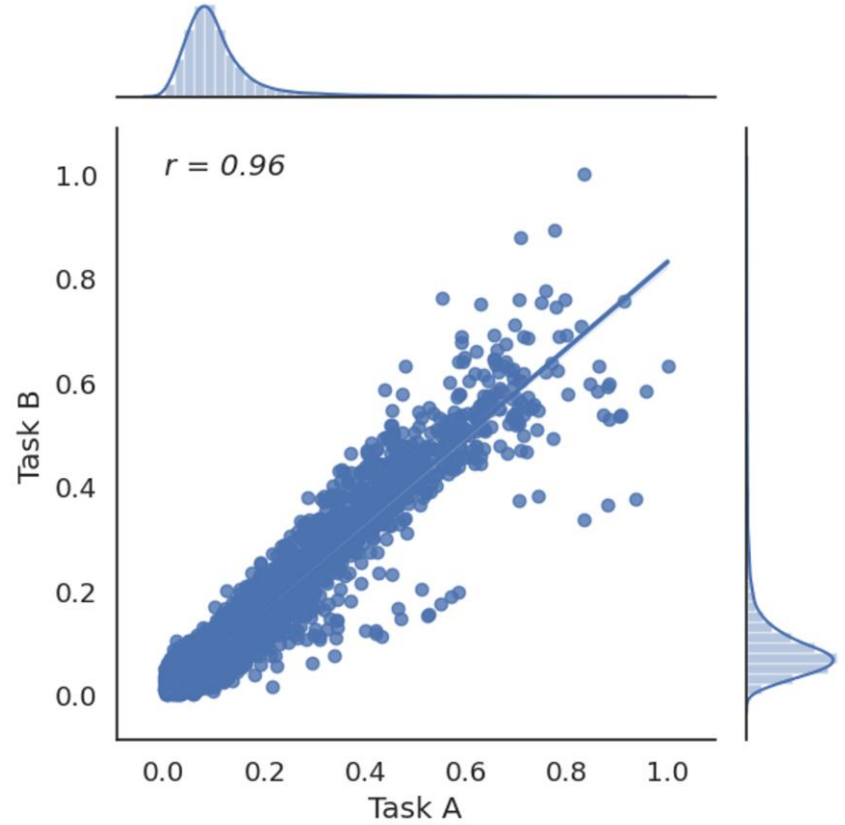
- Goal: measurement the degree of liver toxicity of drug-treated samples
- Proposed liver toxicity distance to measure hepatotoxicity of drug-induced samples based on distance from potentially toxic samples.
  - Constructing toxic space by kernel PCA with RBF kernel



Ruler for a liver toxicity distance

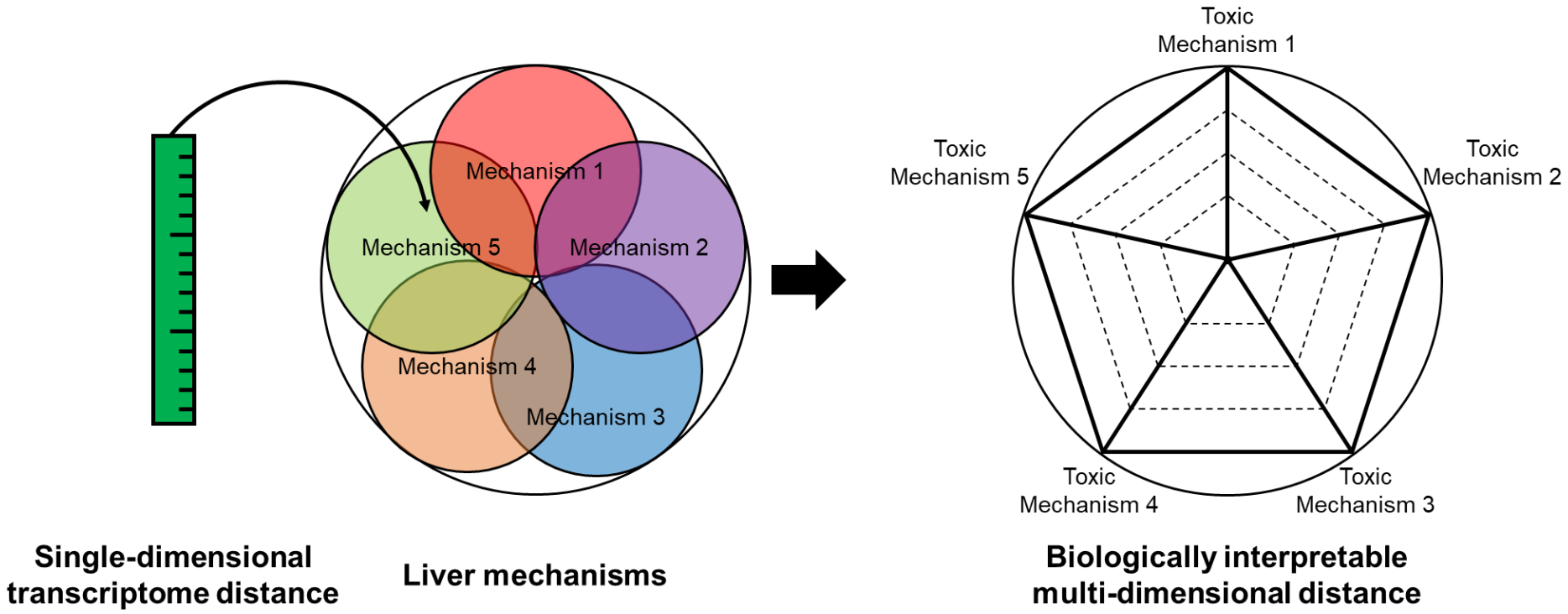
- Goal: measurement the degree of liver toxicity of drug-treated samples
- Distance cross-validation





Step 3: Biological Mechanisms for Liver Toxicity

- Because liver damage can occur through various factors and processes, it is necessary to consider the heterogeneity of the mechanism of liver injury.
- Here, we propose a **biologically interpretable multi-dimensional distance**



Knowledge-based approach for Toxic MoA

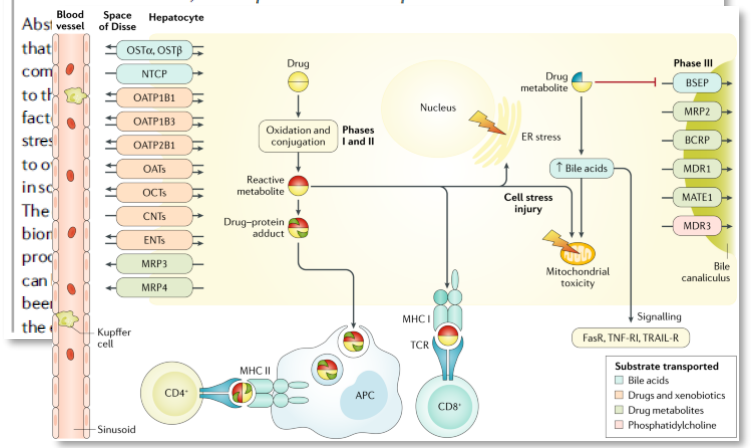
- Literature search to identify well-known liver injury mechanisms

Nature Reviews Disease Primers
ANDRADE, Raul J., et al., 2019

PRIMER

Drug-induced liver injury

Raul J. Andrade[1,2]*, Naga Chalasani[3], Einar S. Björnsson[4,5], Ayako Suzuki[6,7], Gerd A. Kullak-Ublick[8,9], Paul B. Watkins[10,11], Harshad Devarbhavi[12], Michael Merz[8,13], M. Isabel Lucena[2,14]*, Neil Kaplowitz[15] and Guruprasad P. Aithal[16]



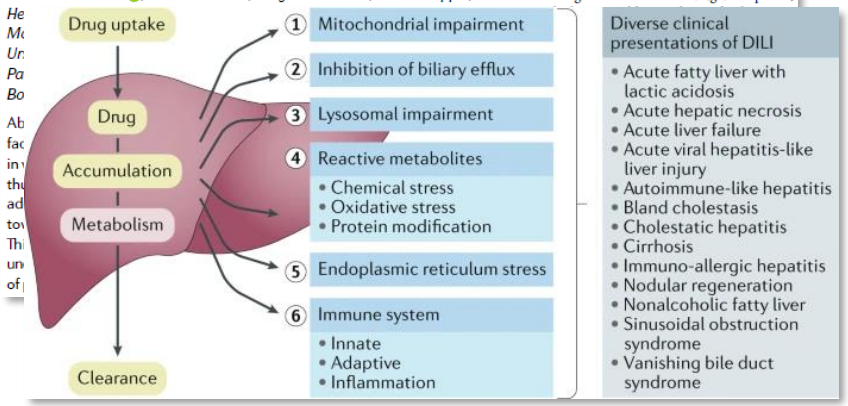
Nature Reviews Drug Discovery
WEAVER, Richard J., et al., 2020

PERSPECTIVES

Managing the challenge of drug-induced liver injury: a roadmap for the development and deployment of preclinical predictive models

Richard J. Weaver, Eric A. Blomme, Amy E. Chadwick, Ian M. Copple,

Given the multifactorial mechanisms of DILI, which contribute to drug attrition in development and in clinical practice, there is a need for new thinking in terms of the development of a holistic approach to the early detection of chemical liabilities which are predictive of DILI. Such an approach must be mechanism-based, pragmatic and sufficiently adaptable to be of practical application: to influence drug design early enough in the discovery phase; and to manage risk assessment in drug development,



Journal of hepatology
HAN, Hui, et al., 2020

Review
JOURNAL OF HEPATOLOGY

Danger signals in liver injury and restoration of homeostasis

Hui Han[1], Romain Desert[1,†], Sukanta Das[1,†], Zhuolun Song[1,†], Dipti Athavale[1,†], Xiaodong Ge[1,†], Natalia Nieto[1,2,*]

Summary
Damage-associated molecular patterns are signalling molecules involved in inflammatory responses and restoration of homeostasis. Chronic release of these molecules can also promote inflammation in the context of liver disease. Herein, we provide a comprehensive summary of the role of damage-associated molecular patterns as danger signals in liver injury. We consider the role of reactive oxygen species and reactive nitrogen species as inducers of damage-associated molecular patterns, as well as how specific damage-associated molecular patterns participate in the pathogenesis of chronic liver diseases such as alcohol-rel... discuss th... transplant... patterns h...

Keywords: Alcohol-related liver disease; Hepatocellular carcinoma; Liver fibrosis; Liver transplantation; Non-alcoholic steatohepatitis; Oxidative stress.

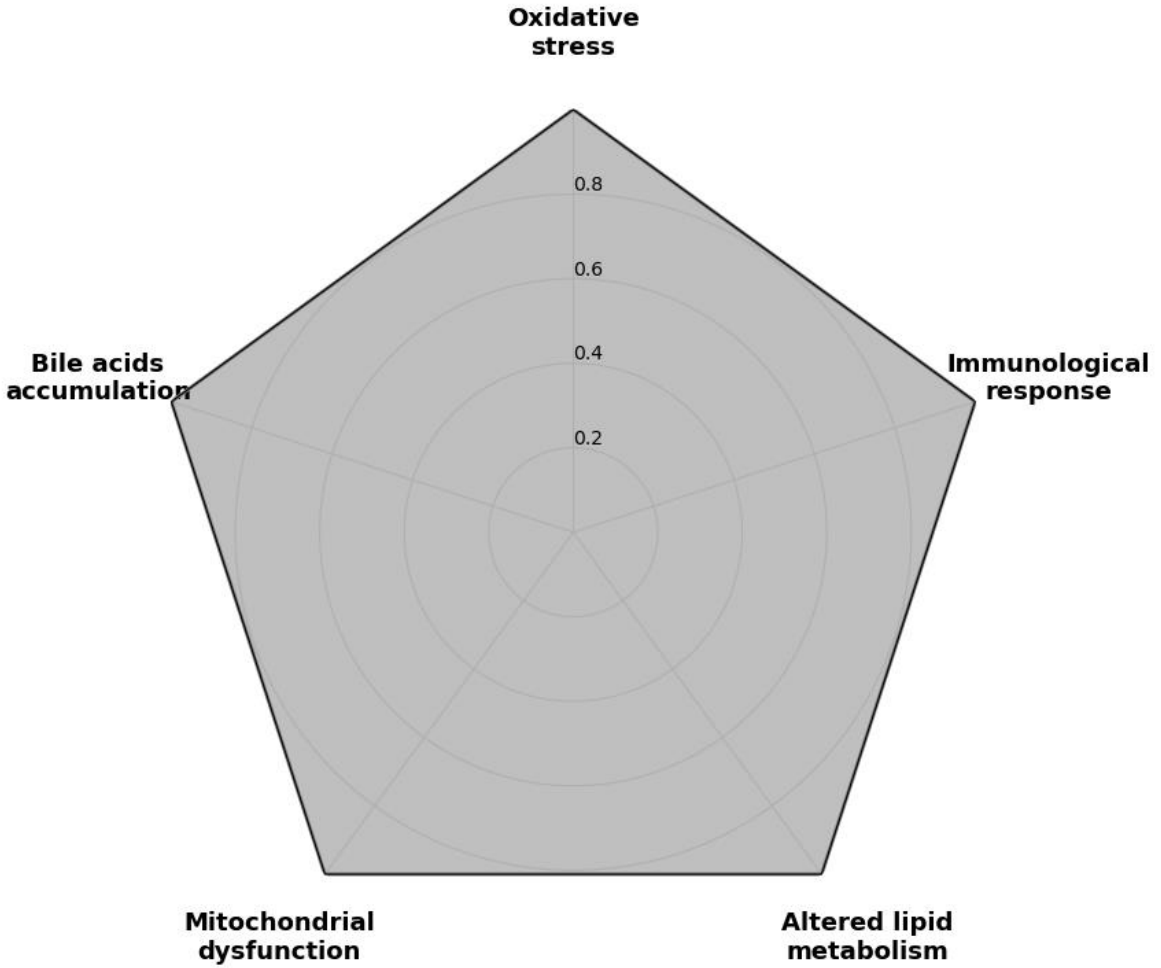
Table 1. ROS and RNS induce events involved in chronic liver disease.

	Effect(s)	Reference(s)
ALD		
ROS	Mitochondrial dysfunction; Proinflammatory; Profibrogenic	51,76,77
RNS	$ONOO^-$ induced liver injury	22,60,61
NASH		
ROS	Lipid peroxidation; Proinflammatory	43,44,69,71
RNS	*De novo* lipogenesis; Proinflammatory	45
Fibrosis		
ROS	TGFβ signalling; HSC activation	46,78
RNS	iNOS induces MMP9; DNA damage; Profibrogenic	62,63
HCC		
ROS	Oxidative DNA damage; DNA adducts; Proinflammatory; Oncogenic; Increase telomerase activity, telomere length and HCC tumour growth; Protein oxidation	47–49
RNS	iNOS promotes HCC stem cell phenotype	32

ALD, alcohol-related liver disease; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma.

Use Prior-Knowledge for Toxic MoA

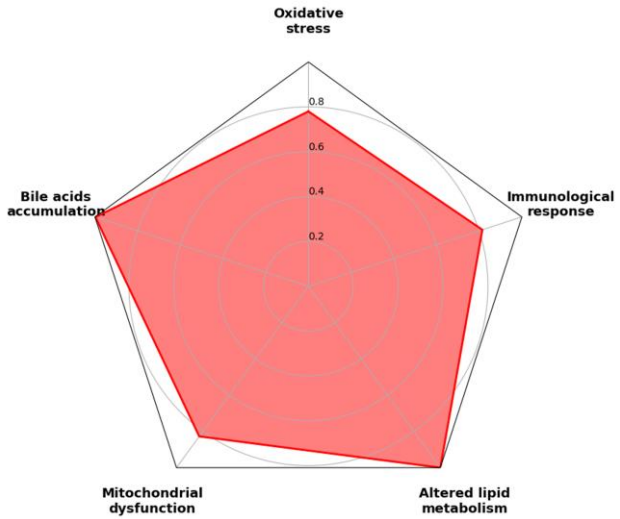
- 5-dimensional distance
  - Oxidative stress **with 6 pathways**
  - Immunological response **with 3 pathways**
  - Altered lipid metabolism **with 13 pathways**
  - Mitochondrial dysfunction **with 2 pathways**
  - Bile acids accumulation **with 2 pathways**



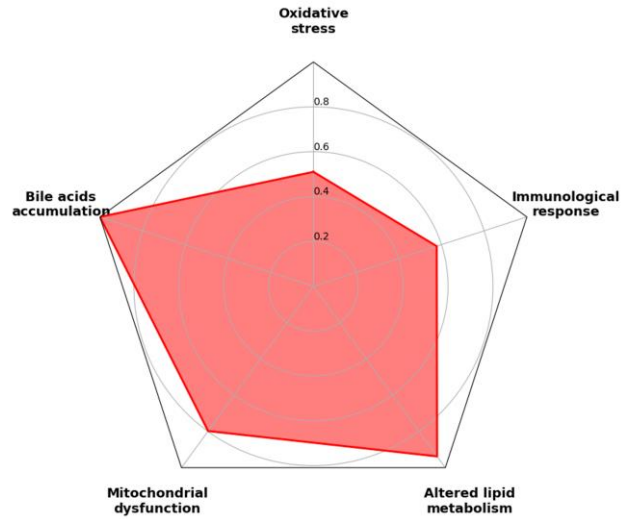
Multi-dimensional Distance Examples

- Potential toxic samples

Drug	Troglitazone
Cell line	VCAP
Time	24hour
Dose	0.1uM



Drug	Dactinomycin
Cell line	HCC515
Time	24hour
Dose	10uM

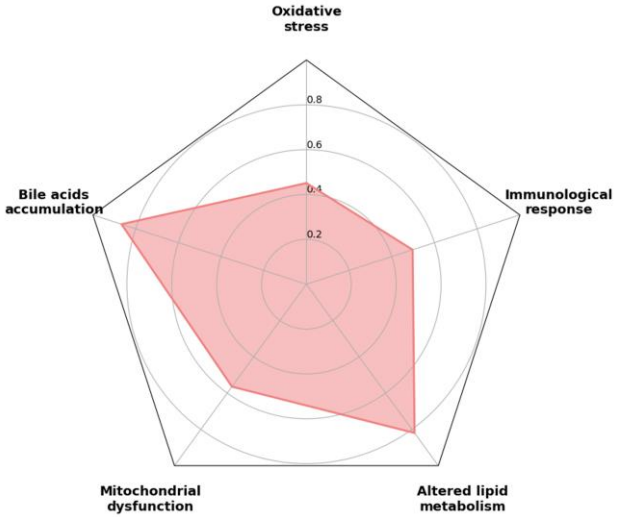


Drug	Doxorubicin
Cell line	PC3
Time	24hour
Dose	10uM

Multi-dimensional Distance Examples (cont'd)

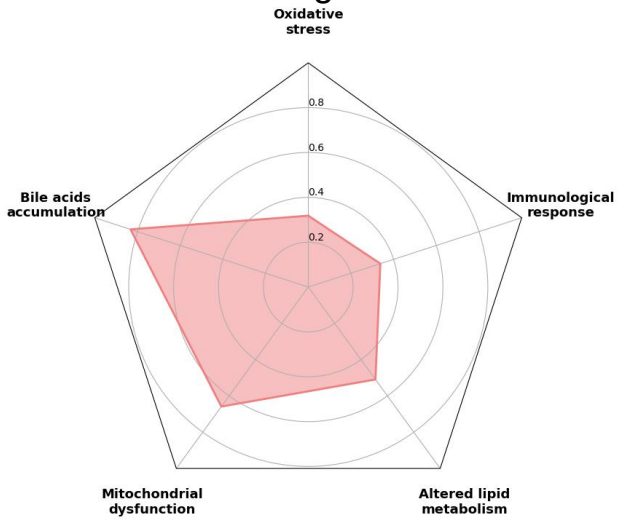
- Non-Potential toxic samples

*Toxic labeled drug



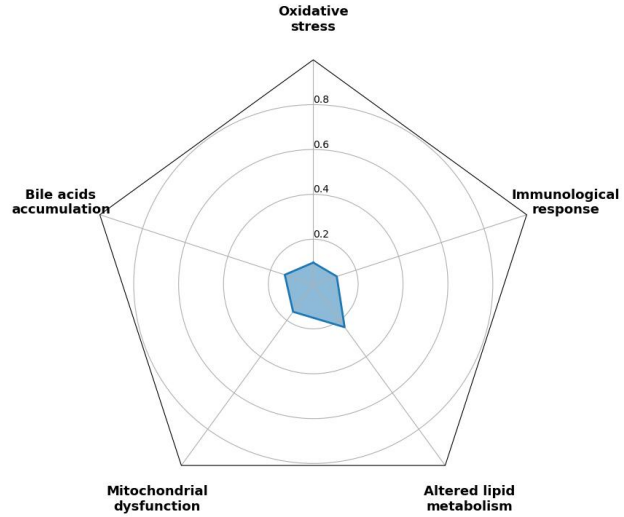
Drug	Dactinomycin
Cell line	HA1E
Time	24hour
Dose	10uM

*Toxic labeled drug



Drug	Amodiaquine
Cell line	PC3
Time	6hour
Dose	10uM

*Non-toxic labeled drug



Drug	Daunorubicin
Cell line	HEPG2
Time	24hour
Dose	10uM

Privileges of Working with Talented People

THANK YOU!!

감사합니다!