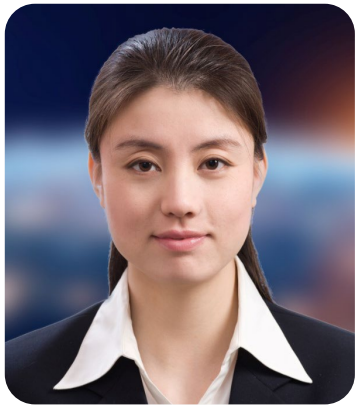
U.S. FOOD & DRUG ADMINISTRATION

Tox-GAN: An Artificial Intelligence Approach Alternative to Animal Studies

— A Case Study With Toxicogenomics



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Division of Bioinformatics and Biostatistics

National Center for Toxicological Research

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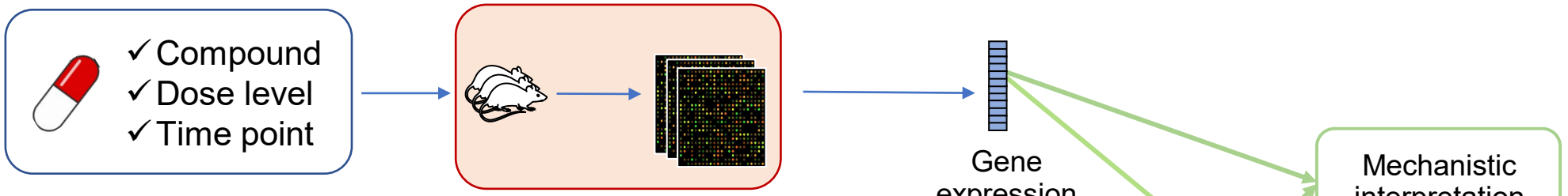
Background

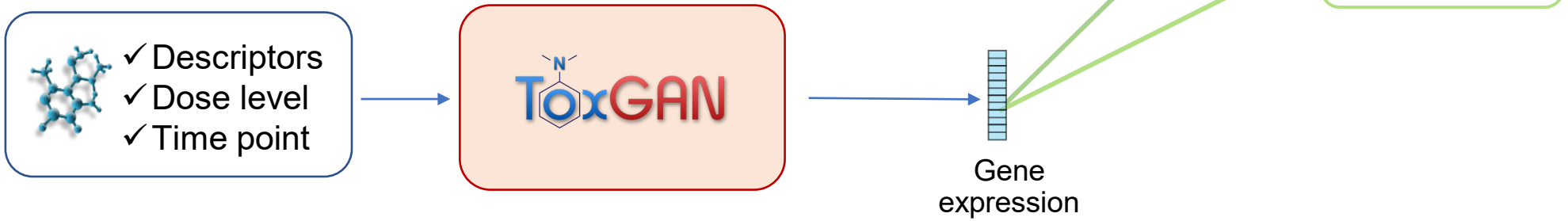
- Animal studies are unavoidable in evaluating chemical and drug safety. Toxicogenomics (TGx) incorporates genomic technologies into conventional animal models, has been widely applied in two areas: improving the understanding of toxicity mechanisms, and enhancing predictive toxicology.
- The FDA Modernization Act 2.0 was recently signed into law by the President, which emphasizes the need to explore alternative testing methods that support the 3Rs principle (Replacement, Reduction and Refinement of animal use).

Motivation - Finding AI Alternatives to Animal Testing

Conventional Toxicogenomics (TGx) Approaches

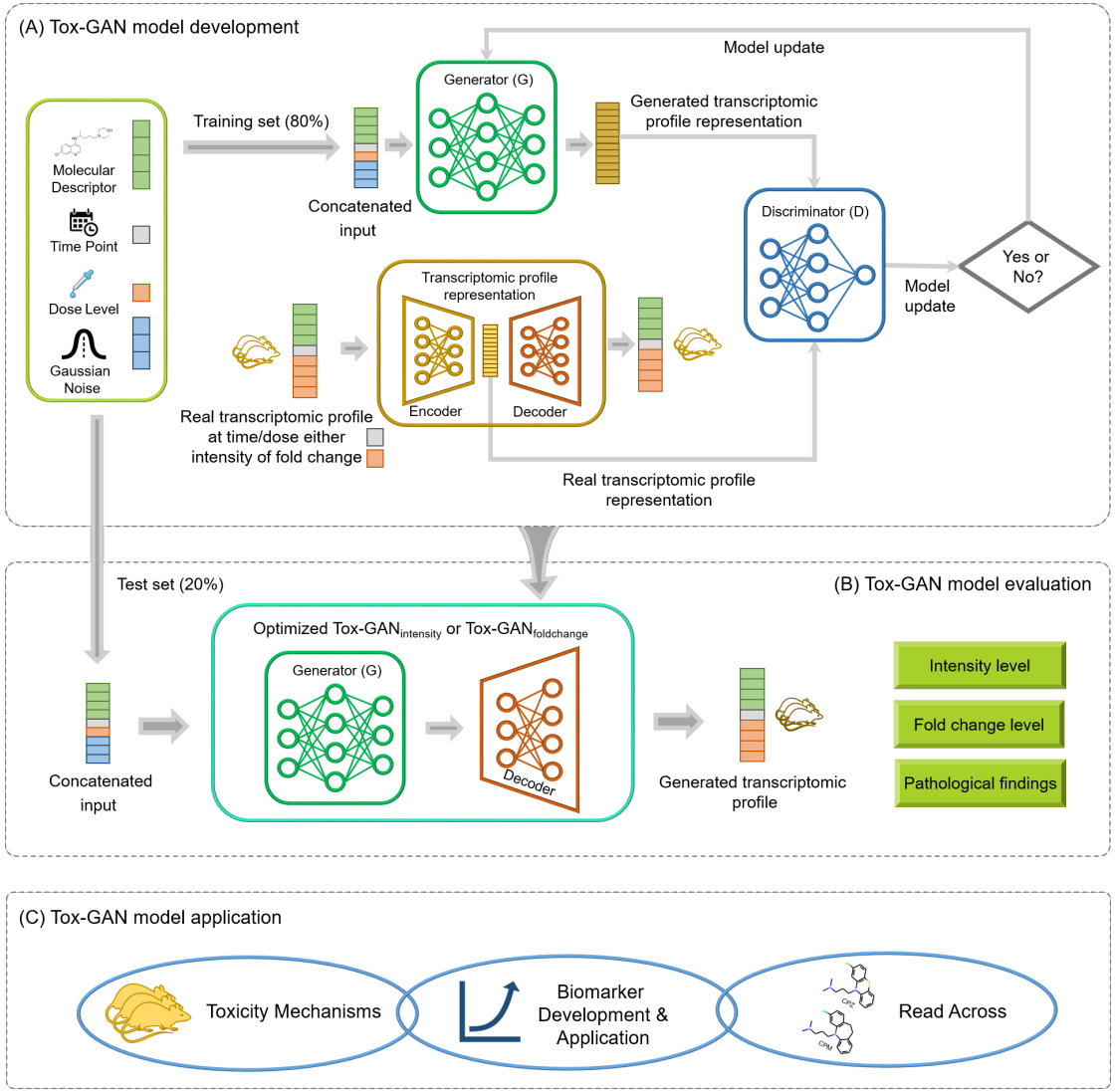


Tox-GAN: Generative AI Alternative to TGx experiment



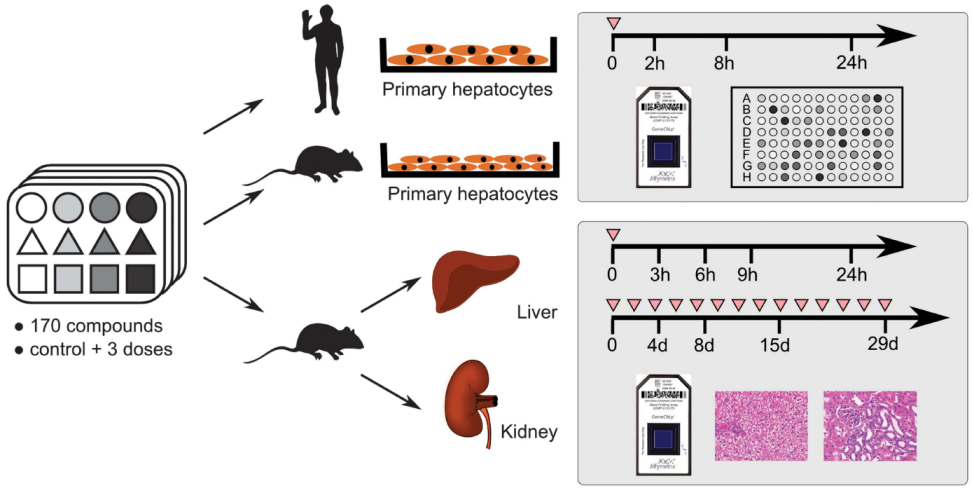
Workflow of the Tox-GAN

- Tox-GAN is a conditional generative adversarial network.
- We implemented Tox-GAN to infer rat liver TGx data from the Open TG-GATEs database.
- Model Applications in:
  1) Toxicity mechanisms
  2) Biomarker development
  3) Read Across



Materials

- Open TG-GATEs:
  - Gene expression: Rat, in vivo, Repeated, Liver: 138 compounds, 4 time points, 3 dose levels
  - Pathological findings

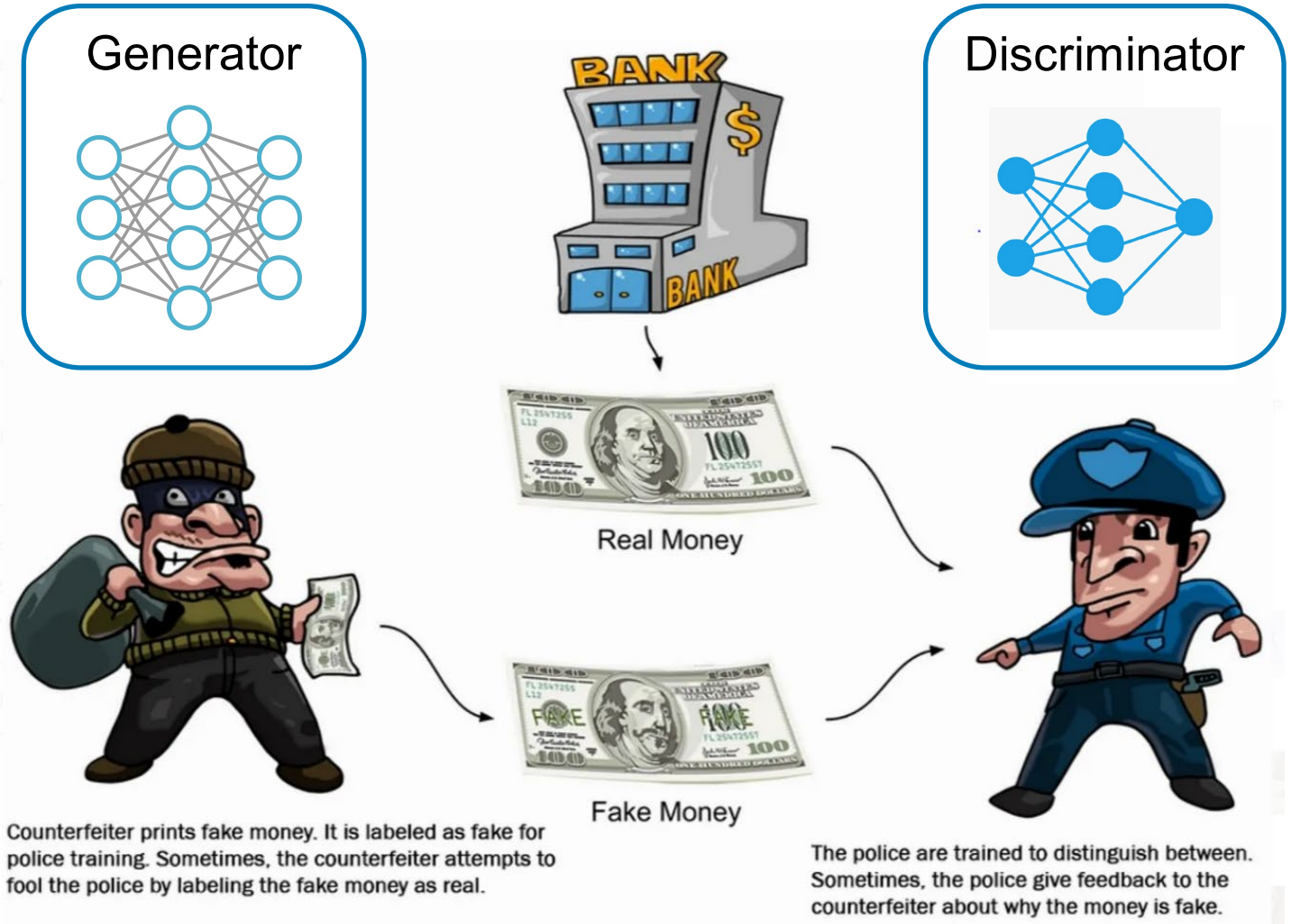


- Chemical Structures
  - Mordred: 1826 molecular descriptors, both 2D and 3D descriptors

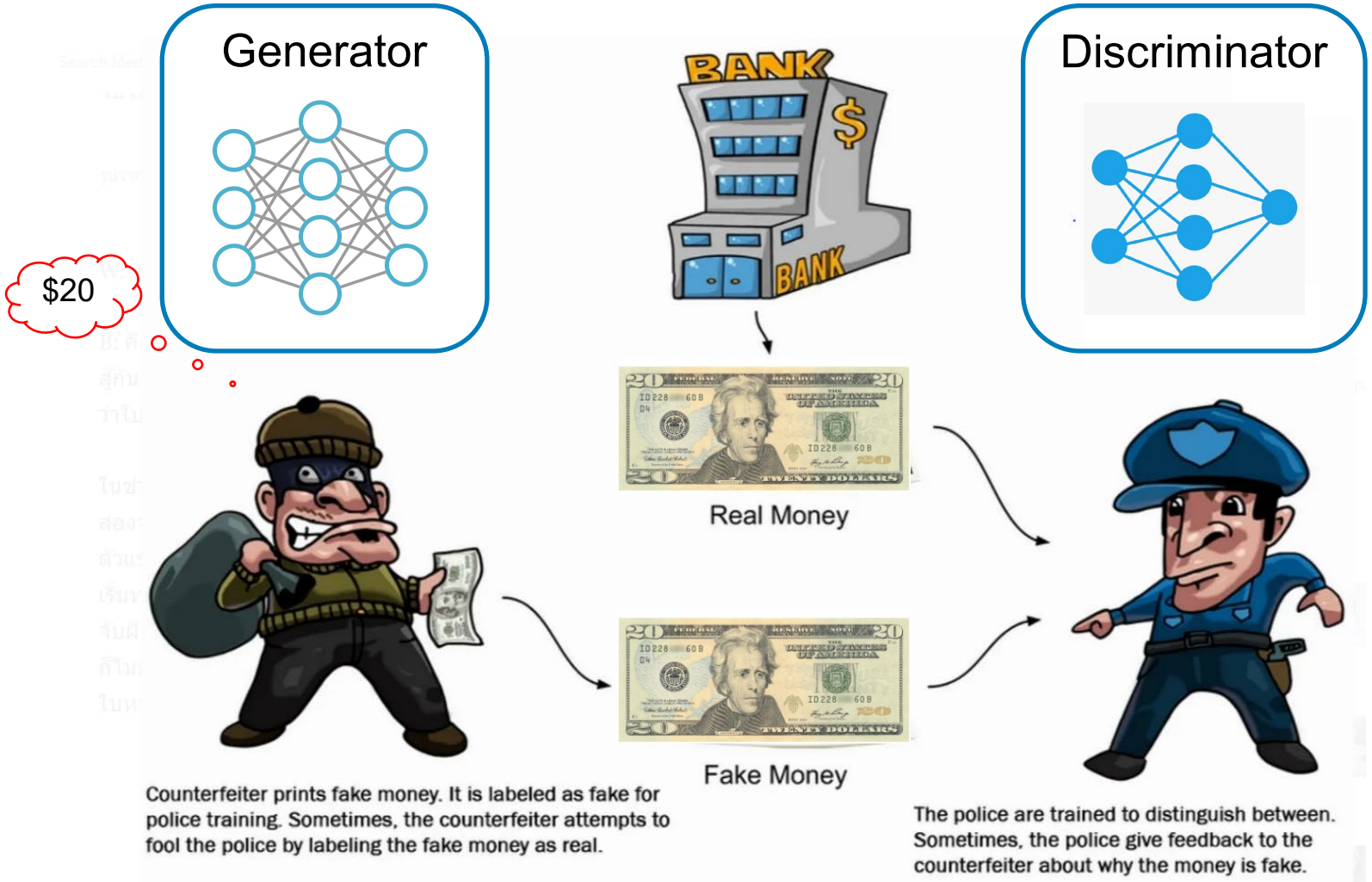
- Moriwaki, H., *et al*., Mordred: a molecular descriptor calculator. *J Cheminform*, 2018. 10(1): p. 4.

- Zhang, J.D., *et al*., Data mining reveals a network of early-response genes as a consensus signature of drug-induced in vitro and in vivo toxicity. *Pharmacogenomics J*, 2014. **14**(3): p. 208-16.

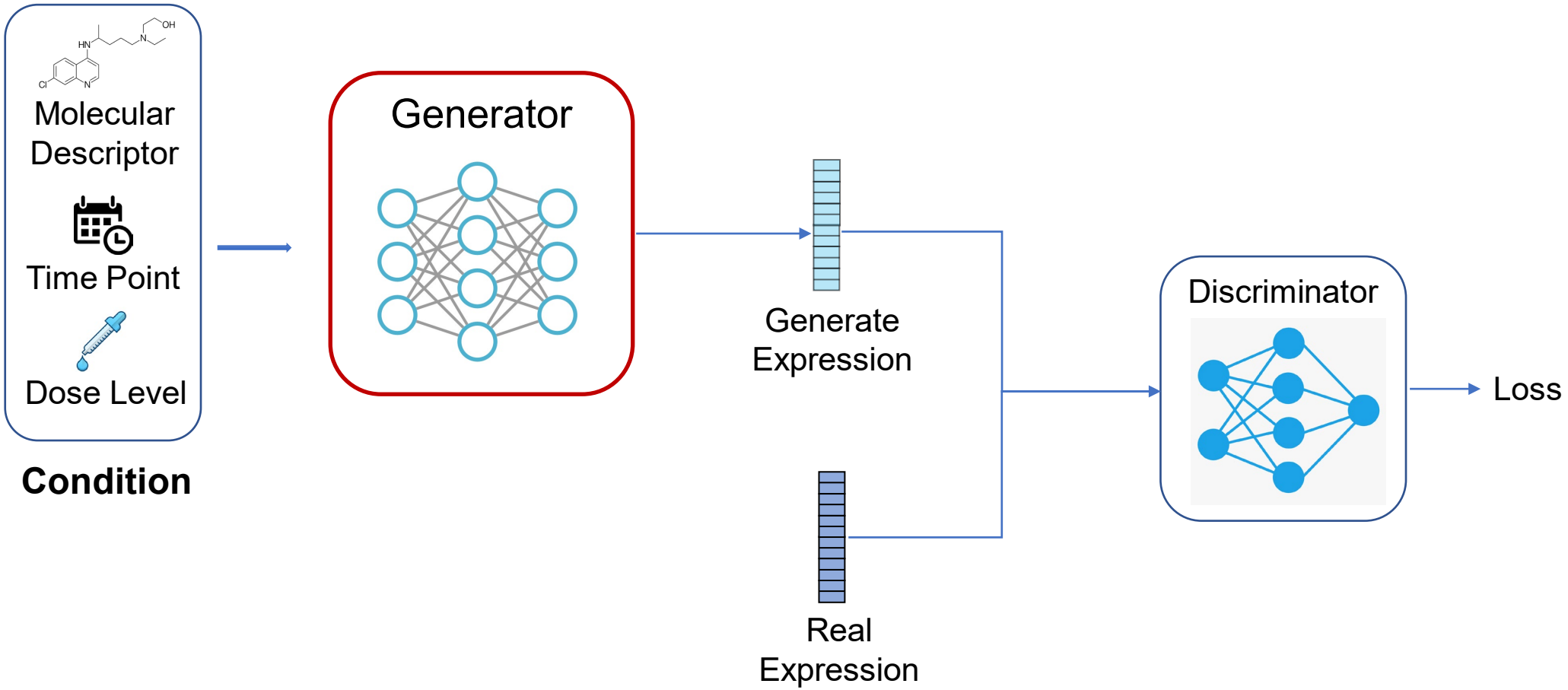
General Idea of Generative Adversarial Networks



Conditional Generative Adversarial Networks

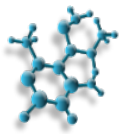


Tox-GAN Model Architecture



Tox-GAN Model Development

138 compounds



- ✓ Descriptors
- ✓ 3 Dose levels
- ✓ 4 Time points
- ✓ 4901 profiles

Training set

110 compounds
For each compound:
4 time points * 3 dose level * 3 replicates
3909 profiles

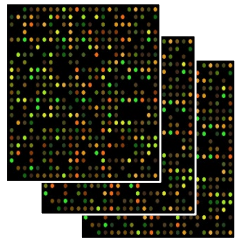
Test set:

28 compounds
For each compound:
4 time points * 3 dose level * 3 replicates
992 profiles

Construct Tox-GAN

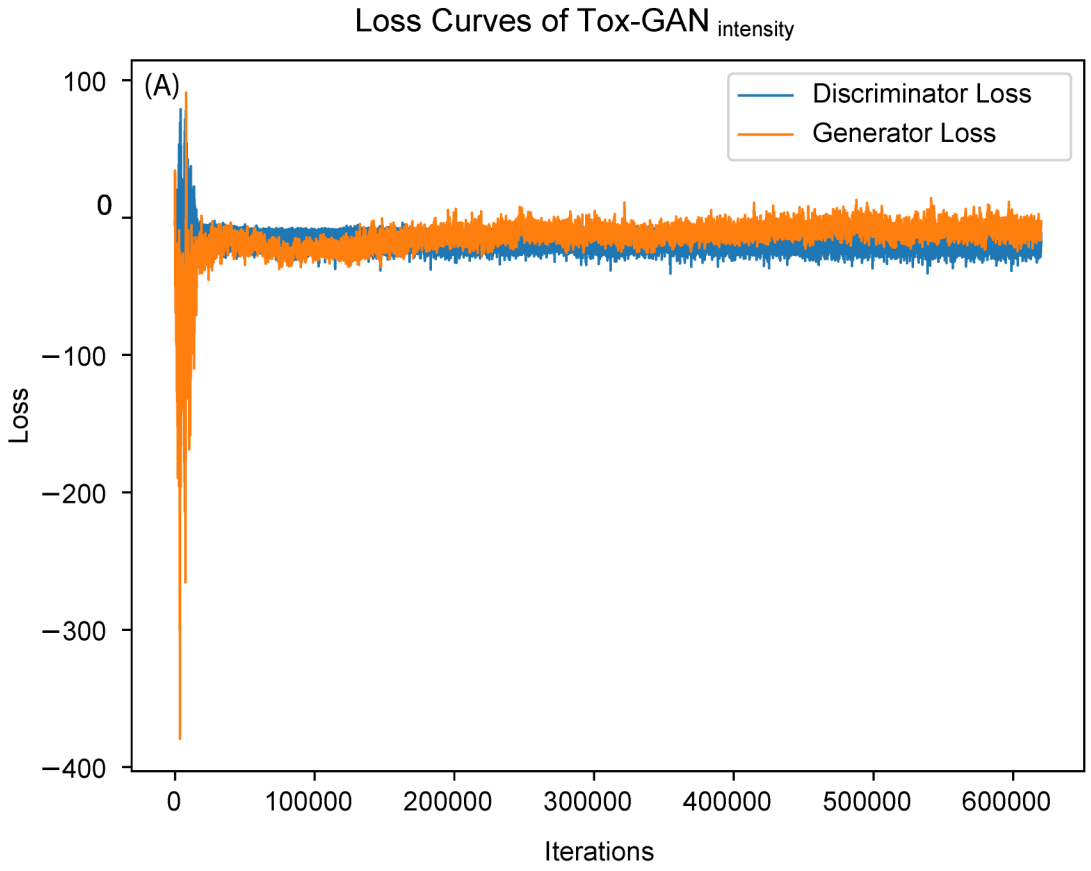
Generator

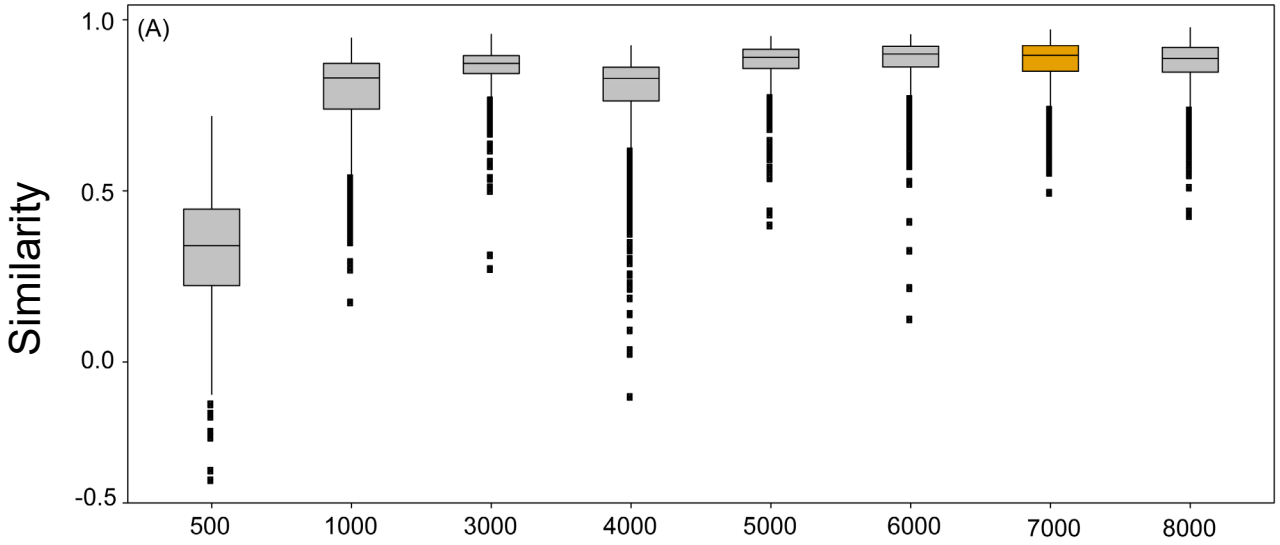
Discriminator



Generated gene expression data for treatments in the training set

Tox-GAN Model Development





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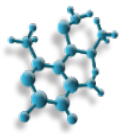

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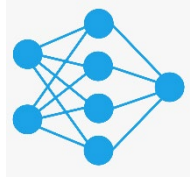
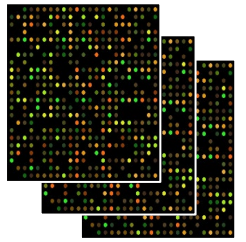


Checkpoint
7000 epoch



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Tox-GAN Model Development

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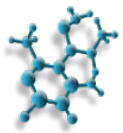

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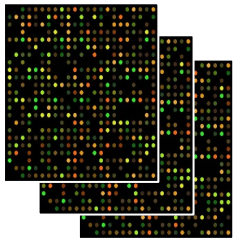
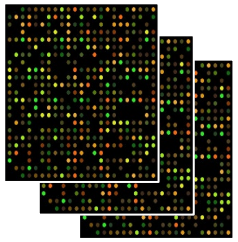
Construct Tox-GAN

ToxGAN



Generated gene expression data for treatments in the training set

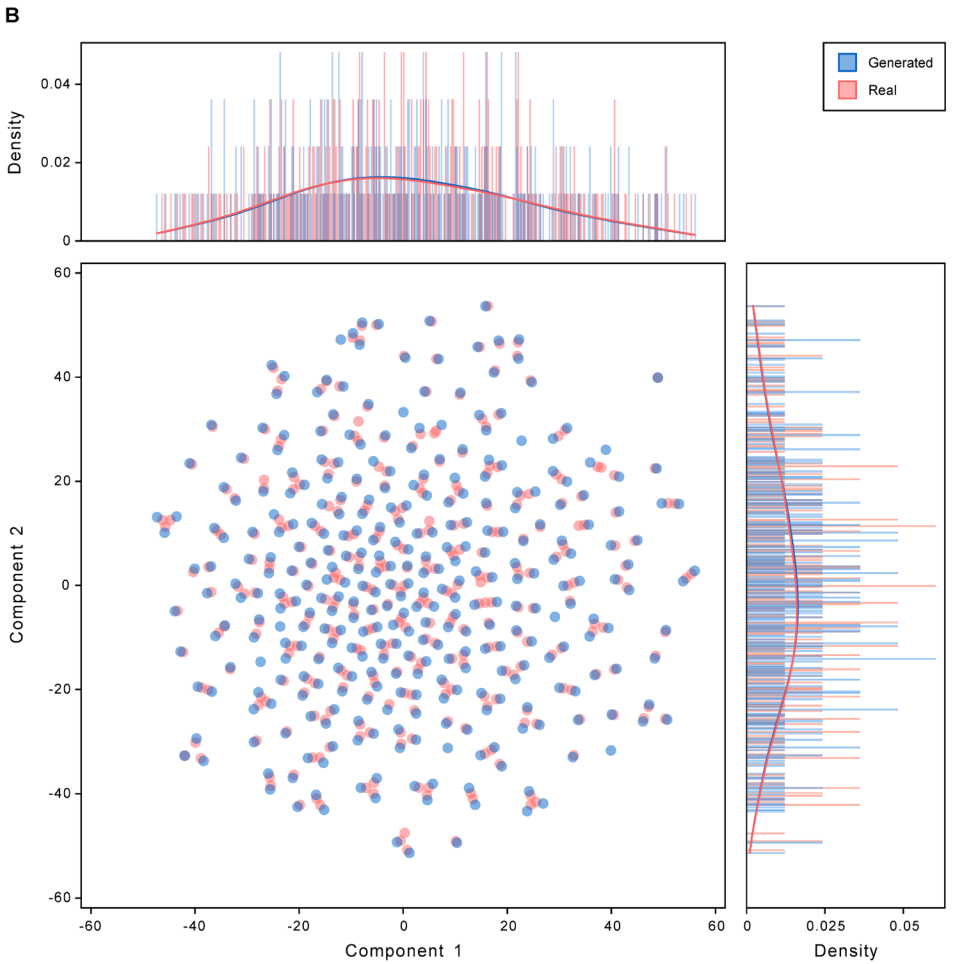


Generated gene expression data for treatments in test set

Tox-GAN Model Evaluation

- Tox-GAN model-produced gene expression fit almost the same distribution as the animal model-profiled gene expression.

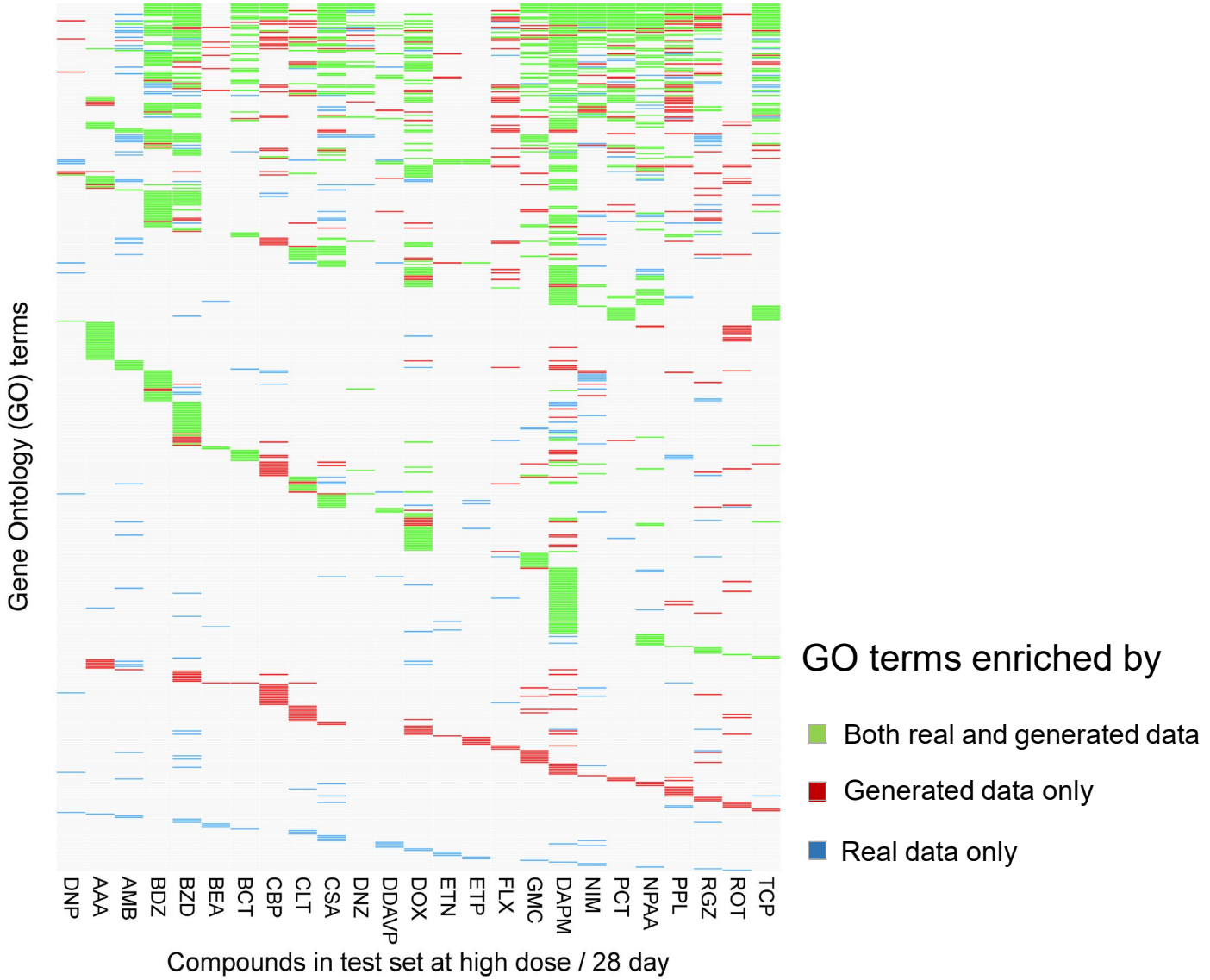
- Furthermore, the average and standard deviation of Pearson correlation coefficients between the generated transcriptomic profiles and their corresponding real ones are 0.997 ± 0.002.



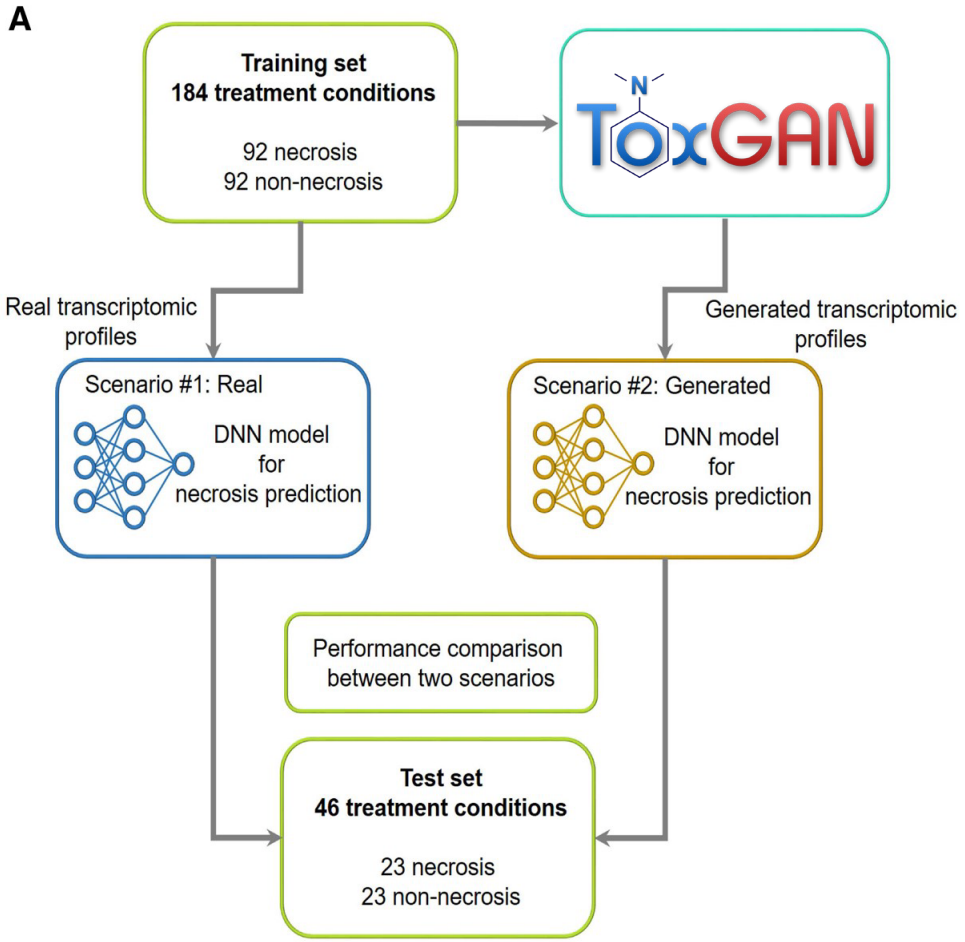
Tox-GAN facilitating understanding of toxicity mechanisms

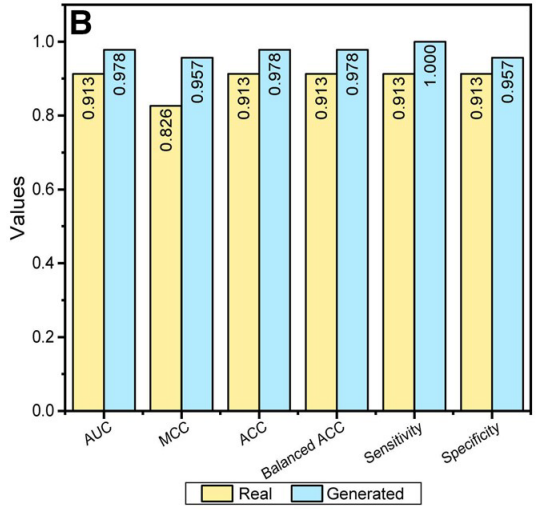
Functional concordance between real and generated gene expression profiles on 28-day repeated high dose treatment study

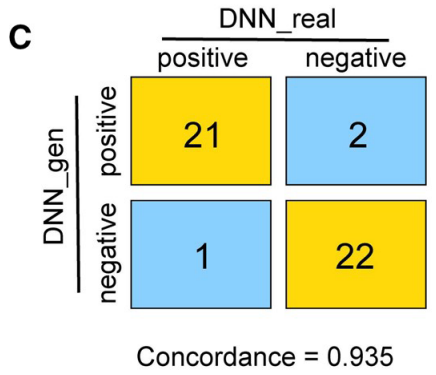
Over 87% agreement in Gene Ontology enrichment analysis was found between the Tox-GAN generated data and animal model profiled data



Tox-GAN enhancing biomarker development

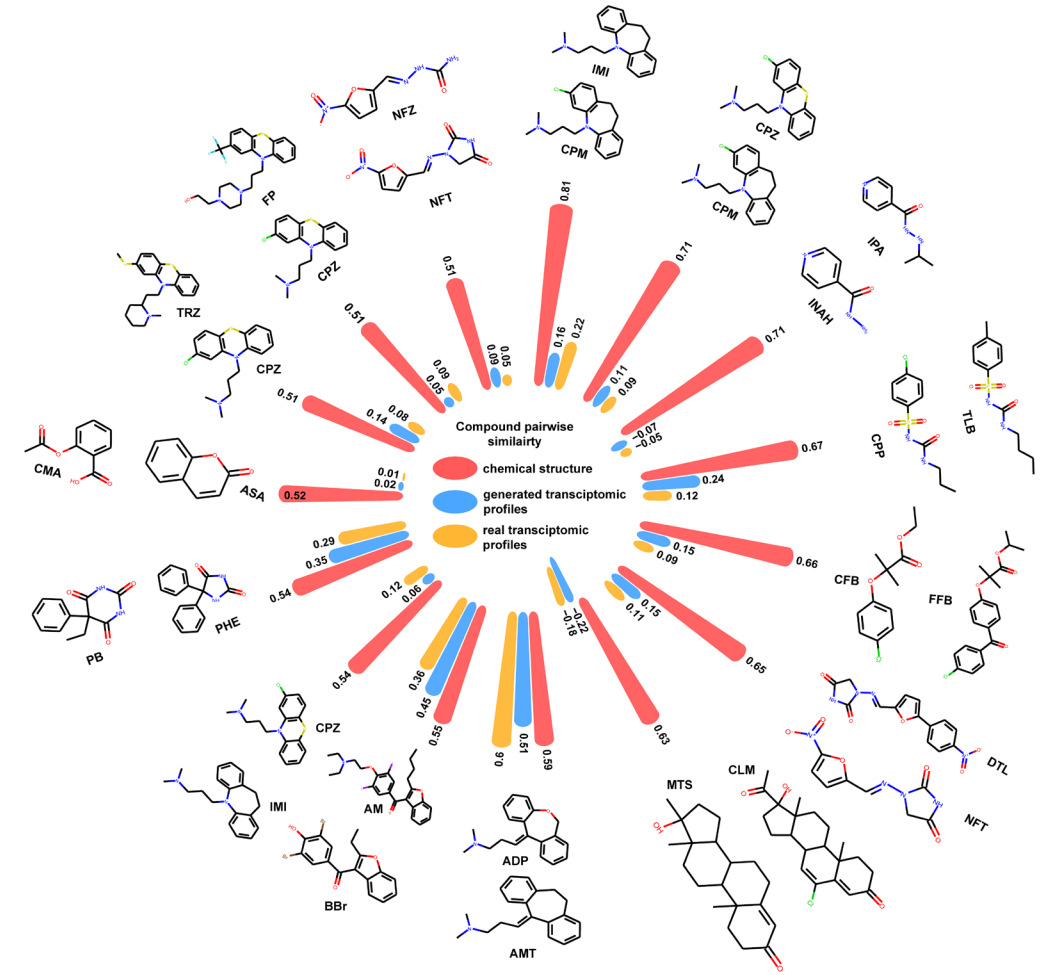






Tox-GAN aiding the read-across

Similarities between the top 15 drug pairs in chemical space, generated transcriptomic profiles and real transcriptomic profiles, respectively.



clomipramine: CPM imipramine: IMI chlorpromazine: CPZ isoniazid: INAH iproniazid: IPA chlorpropamide: CPP
tolbutamide: TLB clofibrate: CFB fenofibrate: FFB dantrolene: DTL nitrofurantoin: NFT chlormadinone: CLM
methyltestosterone: MTS adapin: ADP amitriptyline: AMT amiodarone: AM benzbromarone: BBr phenobarbital: PB
phenytoin: PHE aspirin: ASA coumarin: CMA thioridazine: TRZ fluphenazine: FP nitrofurazone: NFZ

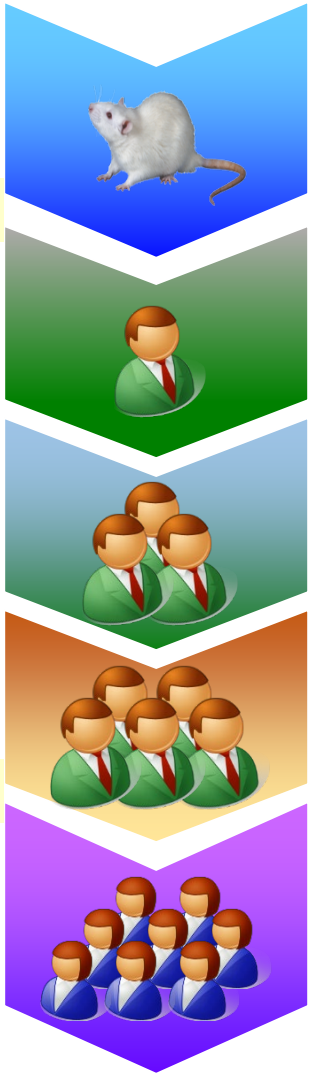
Summary of Tox-GAN Paper

- We proposed a GAN-based framework named Tox-GAN, which learns from the existing animal data and holds promise for generating new animal data just from chemical information in combination of dosage and treatment durations.
- We exemplified the potential utility of Tox-GAN in facilitating the understanding of toxicity mechanisms, enhancing the biomarker development in predictive toxicology, and aiding the chemical-based read-across.
- The developed Tox-GAN models were openly accessible through https://github.com/XC-NCTR/Tox-GAN, which could be utilized for estimating liver transcriptomic profiles of rats treated following experiment protocols used in the Open TG-GATEs in vivo studies.
- This work has been published. For more details, please refer to our paper: **Chen, X., Roberts, R., Tong, W., & Liu, Z. (2022). Tox-GAN: An Artificial Intelligence Approach Alternative to Animal Studies-A Case Study With Toxicogenomics. *Toxicol Sci, 186*(2), 242-259. doi:10.1093/toxsci/kfab157**

Beyond Traditional Testing: The Advantages of Generative AI

- One of the arguments against today's conventional testing methods is that animal studies do not always reflect outcomes in humans. We speculate that the small sample size used in animal studies may also contribute to the poor translation.
- For generative AI model, we can generative testing results with a large population of animals, offering a potential venue to assess rare adverse events in the human population which are unlikely to be detected in conventional animal studies.

Beyond Traditional Testing: The Advantages of Generative AI



Idiosyncratic liver toxicity in human

It is widely acknowledged that Phase III trials in drug development may not reliably predict rare adverse events, such as iDILI. This is mainly due to the limited and controlled population sample in these trials, which may not be representative of the real-world population. However, when it comes to explaining the poor translation of animal study results to humans, the main factor often cited is species differences in biology. Despite this, we speculate that the small sample size used in animal studies may also contribute to the poor translation.

Beyond Traditional Testing: The Advantages of Generative AI

- AnimalGAN: A Generative Adversarial Network Model Alternative to Animal Studies for Clinical Pathology Assessment, available in BioRxiv.
- We carried out a 28-day study with a large population of rats for DILI assessment of three thiazolidinediones. These three drugs are similar in chemical structure (share the same scaffold) but with different DILI risk. The virtual experiment with 100,000 rats revealed the difference in DILI potential among these three. If we estimated the DILI frequency as a percentage of rats meeting the overall DILI risk criteria, the results agreed with the iDILI frequencies in the human population of these three thiazolidinediones; that is 1.9% (troglitazone), 0.26% (pioglitazone), 0.25% (rosiglitazone). The results offer a potential venue to assess rare adverse events in the human population which are unlikely to be detected in conventional animal studies.

Criteria	Troglitazone	Pioglitazone	Rosiglitazone
ALT>ULN*	1230	1820	1467
AST>ULN	7413	4315	4591
TBIL>ULN	3421	2083	2215
ALT>ULN or AST>ULN, and TBIL>ULN	375	161	158

The number of rats exhibiting drug-induced liver injury estimated by AnimalGAN for the three thiazolidinediones under the 28-day study with high dose in 100,000 rats.

Acknowledgement

- Dr. Weida Tong, DBB, NCTR, FDA
- Dr. Zhichao Liu, Boehringer Ingelheim
- Dr. Ruth Roberts, ApconiX Ltd and University of Birmingham, UK

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Thank you!

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