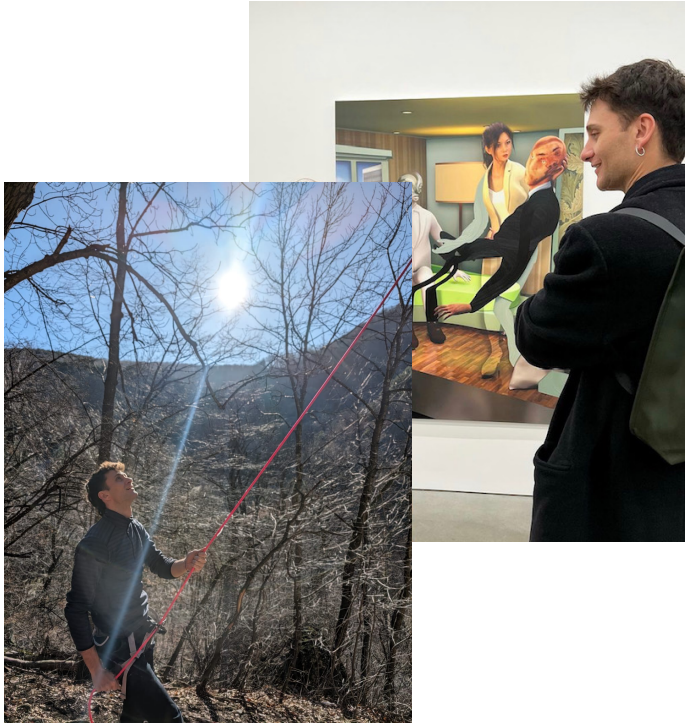
Statistical approach enabling technology-specific assay interference prediction from large screening data sets

04 March 2024

Vincenzo Palmacci – AIDD ESR11

About me



universität wien

Problem statement

-Thanks to their sensitivity and efficiency, **fluorescence-based assays are the most widely employed technology** for the high-throughput-screening (HTS) of compounds [1, 2].

-Despite the technical advantages brought to the field, fluorescence-based assays result in a significant number of **false positive readouts caused by assay interference** [3].

- If false readouts remain undetected, they may **trigger costly follow-up studies** that may eventually turn out as futile.

1. Macarron R. et al. *Impact of high-throughput screening in biomedical research*. Nat. Rev. Drug Discovery
2. https://atrandi.com/blog/fluorescence-assays-dominate-life-science-research
3. Sink R. et al. *False positives in the early stages of drug discovery.* Curr. Med Chem

Fields of application

-HITS TRIAGING:

The practice of selecting a compound series with a promising efficacy profile that meets basic safety requirements and to justify investment in its optimization [4].

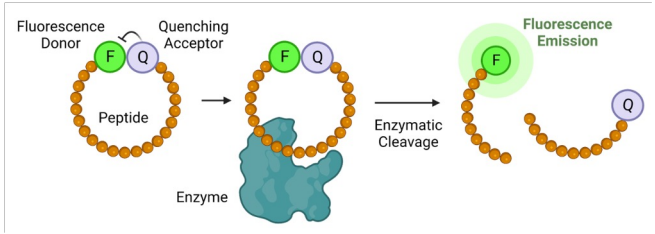
-NEGATIVE DESIGN:

Battery of methods that are usually employed to eliminate molecules with undesired properties [5].

4. Vincent F. et al. *Hit Triage and Validation in Phenotypic Screening: Considerations and Strategies.* Cell Chem Bio
5. Yang Z. et al. *Application of Negative Design To Design a More Desirable Virtual Screening Library.* J. Med Chem

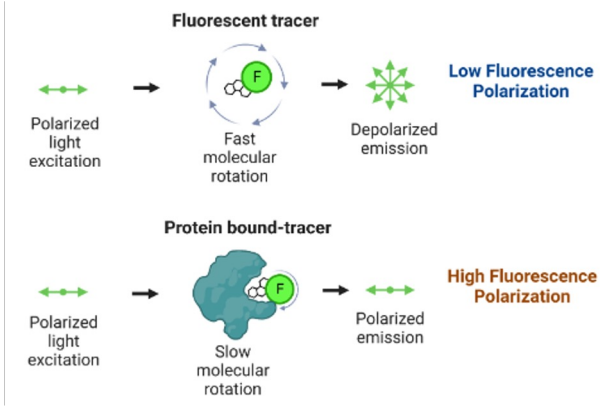
Fluorescence Intensity Assays (FLINT)

Fluorogenic assays



- Convenient for screening enzymatic inhibitors
- Fluorescent emission upon enzymatic cleavage

Fluorescence polarization (FP)



- Detect dynamic interaction between the biological target and the ligand
- Fluorescent emission upon interaction

Credits: https://bpsbioscience.com/

Other popular fluorescence-based assay formats

- Fluorescence Resonance Energy Transfer (FRET)

  Measures the energy transfer between a donor-acceptor pair. For the energy transfer to work donor and acceptor must be in close proximity.

- Time-Resolved FRET (TR-FRET)

  Measures the time a fluorophore spends in the excited state before it reverts to its ground state by emitting a photon (FLT).

Pro of fluorescence-based assays

High specificity.

High sensitivity with low background noise.

Simple operation.

Credits: https://bpsbioscience.com/

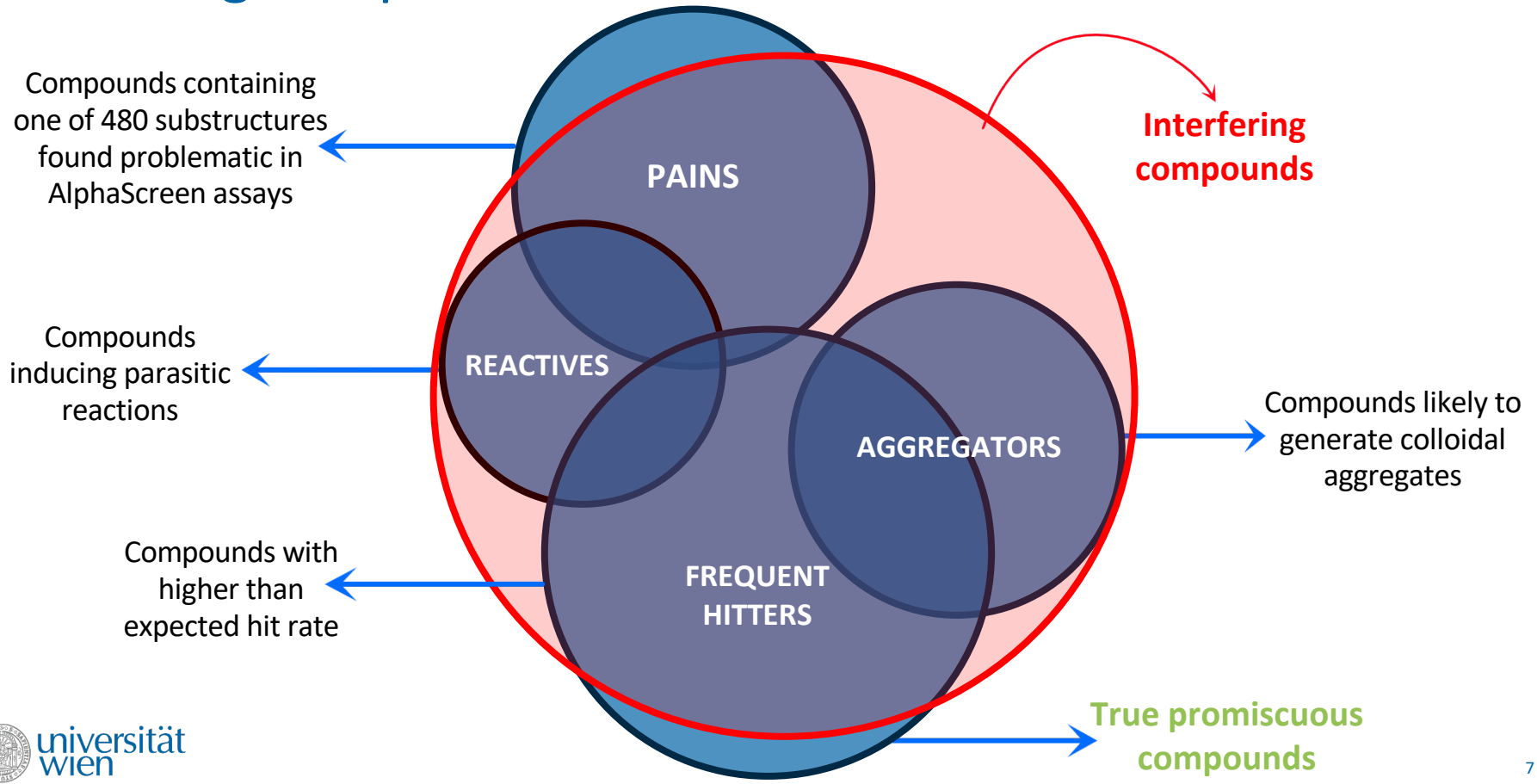
No one’s safe: false positive readouts

“Many hits are artefacts - their activity does not depend on a specific, drug-like interaction between molecule and protein. Artefacts have subversive reactivity that masquerades as drug-like binding and yields false signals across a variety of assays.”[6]

universität wien

6. Baell J. et al. *Chemical con artists foil drug* discovery. Nature

Interfering compounds and interference mechanisms



universität wien

Dealing with assay interference: prevention measures and hit-triaging

Experimental countermeasures

- Screening with non-ionic detergents to prevent compounds aggregation
- Use of novel fluorophores emitting in a different region of the spectrum
- Use orthogonal assays to confirm the primary hits
- Implementation of counter-screen assays to identify interfering compounds

In-silico methodologies

Global methods:

HitDexter3, Pan-Assay interference compounds (PAINS)*

Specialized methods:

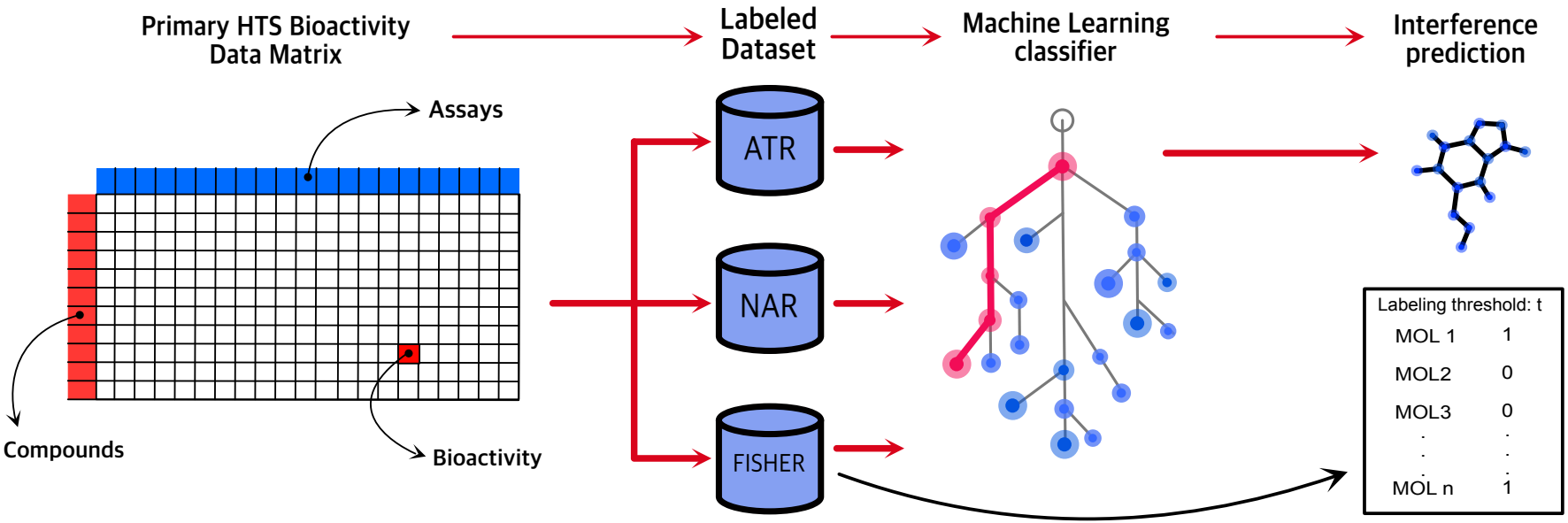
InterPred, ChemFluo, AZ (TR-)FRET interference classifiers

universität wien

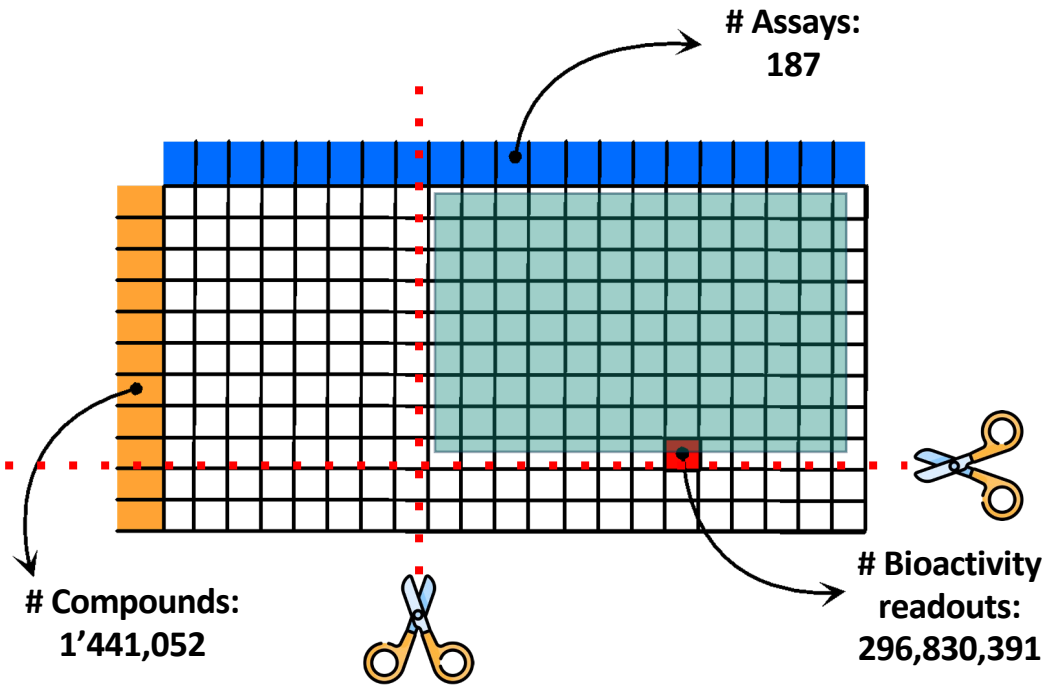
Why we do *in-silico* modelling? To avoid running expensive experiments!
Review coming soon… stay tuned!

A new methodology to identify compounds interfering with specific-assay technologies

Overview


Primary HTS Bioactivity Data Matrix
Labeled Dataset
Machine Learning classifier
Interference prediction
Assays
Compounds
Bioactivity
ATR
NAR
FISHER
Labeling threshold: t
MOL 1 1
MOL2 0
MOL3 0
MOL n 1

Data preprocessing: Bayer AG HTS historical data



Preprocessing pipeline

1. Assays must have bioactivity recorded for at least 80% of the compounds

2. Compounds must have bioactivity recorded for at least 80% of the assays

Dataset composition after step 1 and 2:

205 assays, 1’488’407 compounds

3. Assays must be annotated

4. Compounds must be unique (SMILES strings matching)

5. Binarize Z-scores following experimentalist indications

Note: Readouts were available for both main and background signal

Dataset characterization: Bayer AG HTS historical data

Assays space:

FLINT: 56 Blue , 23 Green, 8 Red

FRET: 16

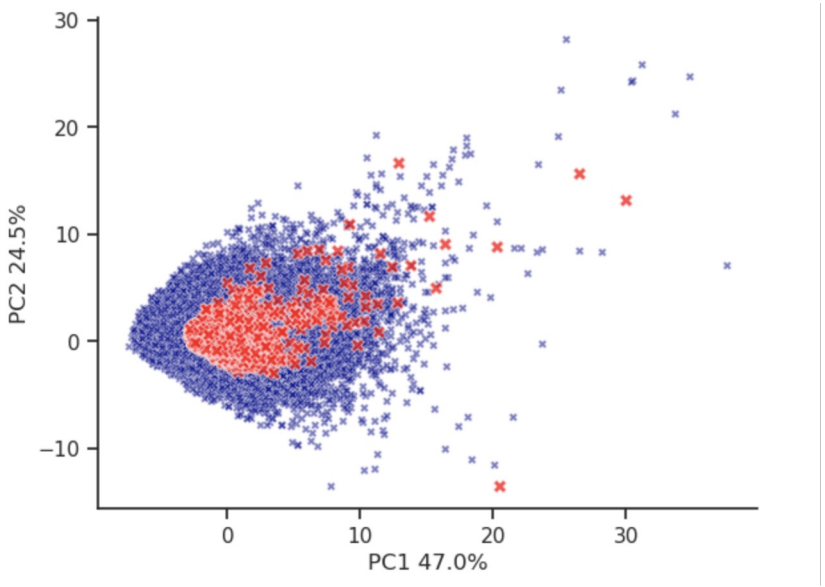
TR-FRET: 10

Bioluminescence: 76

Cell-based: 88

Biochemical: 99

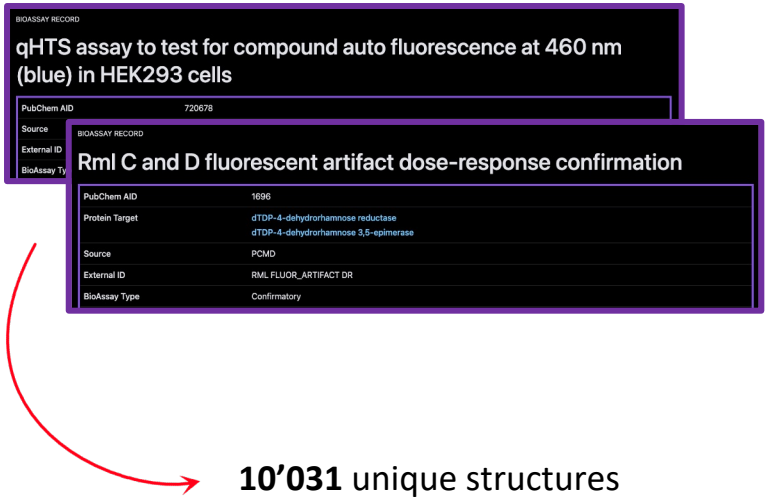
Chemical space



PCA comparing the training set chemical space (BLUE) and the DrugBank approved drugs space (RED)

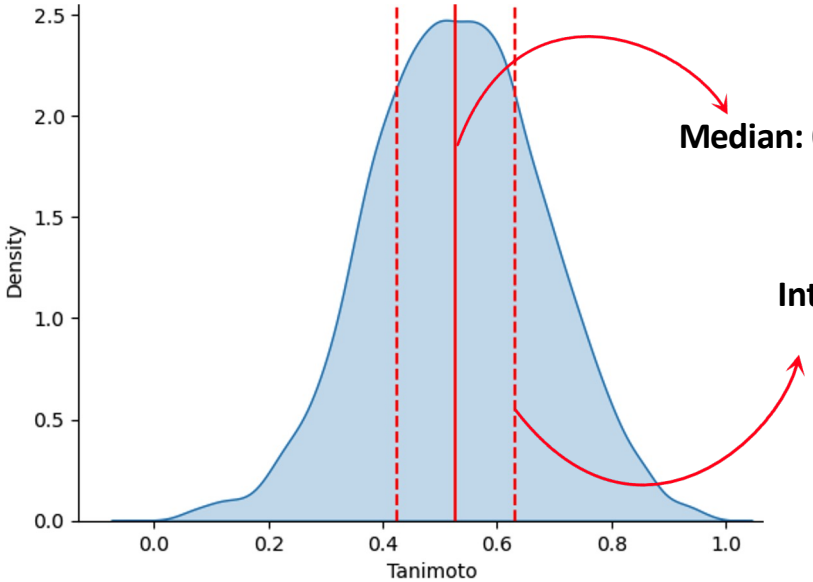
universität wien

Data collection and preprocessing: PubChem test set



10’031 unique structures

Tanimoto coefficient distribution



Distribution of compounds’ maximum Tanimoto coefficient computed between the training and the PubChem test set.

universität wien

Labeling compounds likely to interfere with fluorescence-based assays

Labelling compounds likely to interfere with the assay technology: compute interference metrics

Activity-to-tested ratio (ATR) reloaded

Activity-to-tested ratio is computed as:

$$ATR^i = \frac{\#\ active\ readouts}{\#\ times\ tested}$$

Where i is the i-th compound in the dataset

1. Compute compounds ATR:

- ATR in fluorescence assays
- ATR in other assays

2. Apply threshold to obtain binary interference labels

Noise-to-active ratio (NAR)

Noise-to-active ratio is computed as:

$$NAR^i = \frac{\#\ active\ back \cap \#\ active\ main}{\#\ times\ tested}$$

Where i is the i-th compound in the dataset

1. Compute compounds NAR considering only fluorescent assays

2. Apply threshold to obtain binary interference labels

Fisher exact test

For the compound contingency table X:

	0	**1**
Fluorescent assays	a	b
Other technologies	c	d

1. Compute compounds p-values applying Fisher –exact test

2. Apply threshold to obtain binary interference labels

Labelling compounds likely to interfere with the assay technology: compute binary labels

Thresholds applied to compute binary interference labels

	Percentage of likely interference compounds (thresh)			
	2%	**5%**	**10%**	**20%**
ATR	5.00	3.00	1.00	0.90
NAR	0.10	0.07	0.04	0.03
p-value from Fisher's exact test	0.01	0.07	0.17	0.35

Rationale applied to interference metrics to compute binary labels

$$ATR^{i}_{Fluo} \geq mean(ATR_{Other}) + thresh * std(ATR_{Other})$$

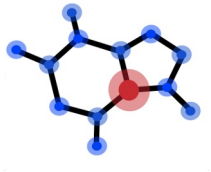
$$NAR^{i} \geq thresh$$

$$p^{i} \leq thresh$$

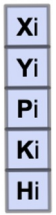
universität wien

Development of machine learning classifiers for assay interference prediction

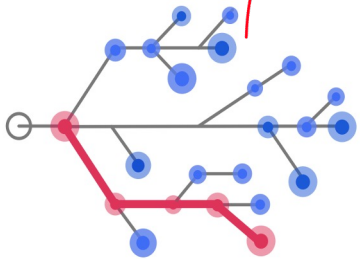
mol



GetMorganFingerprintAsBitVect



Morgan2(nbits=2048)

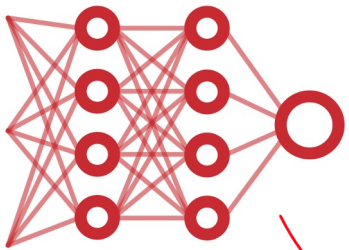


RFC

BalancedRandomForest (imbalanced-learn)
BayesOpt 50 iterations

Hyperparameters optimized:

- n_estimators
- max_depth
- bootstrap



MLP

MLP (PyTorchLightning)

ELU activation function
BinaryCrossEntropyLoss
WeightedRandomSampler

Optuna 50 iterations

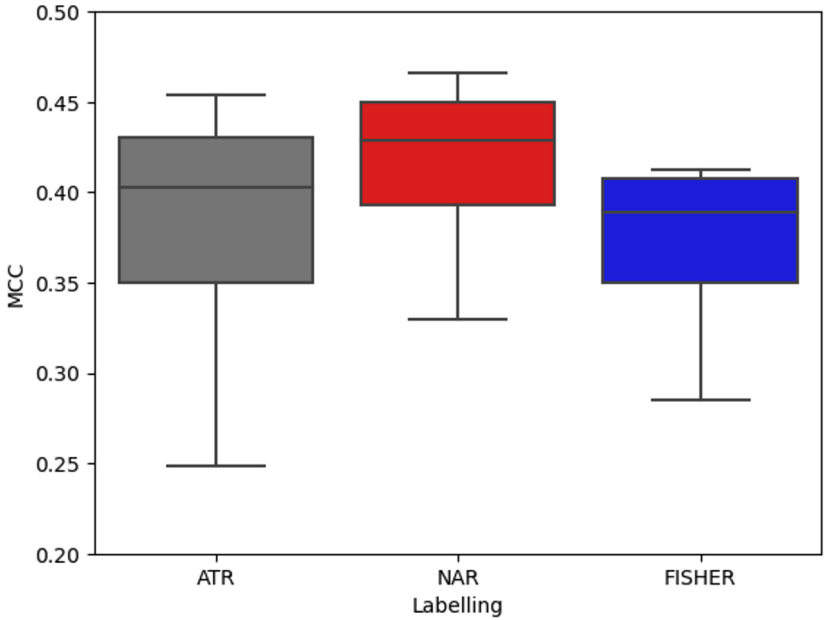
Hyperparameters optimized:

- n_layers
- n_units
- dropout
- learning_rate

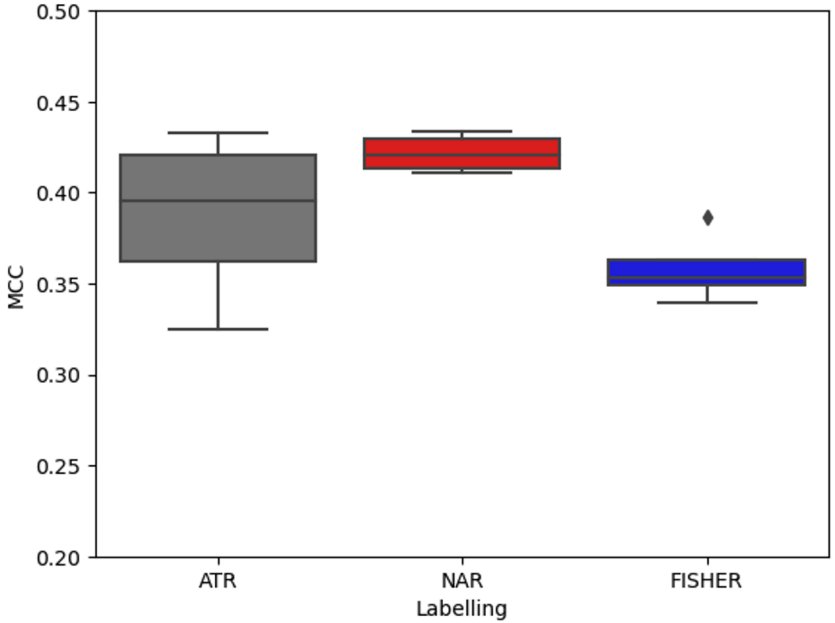
universität wien

Model performances on the Bayer AG test set

RFC MCC for different labelling methods

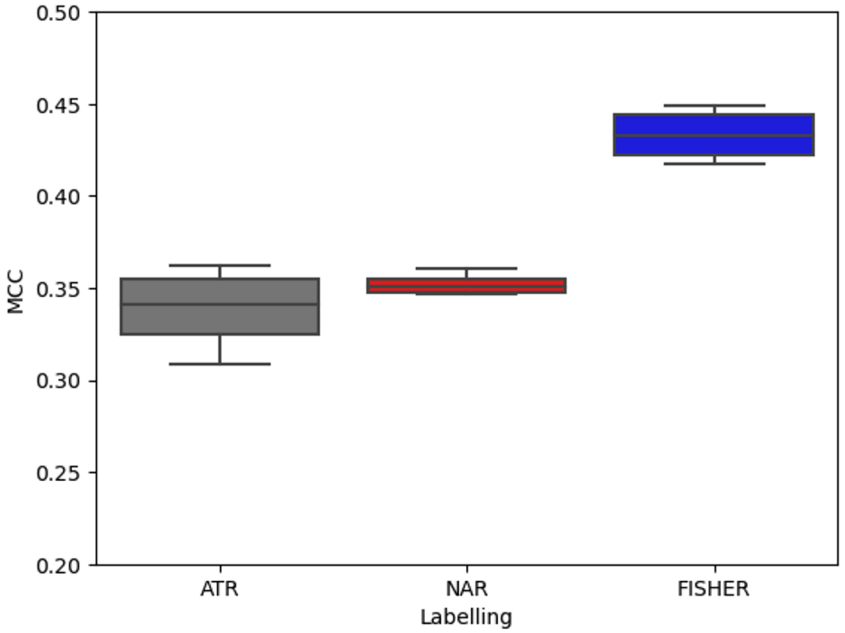


MLP MCC for different labelling methods



universität wien

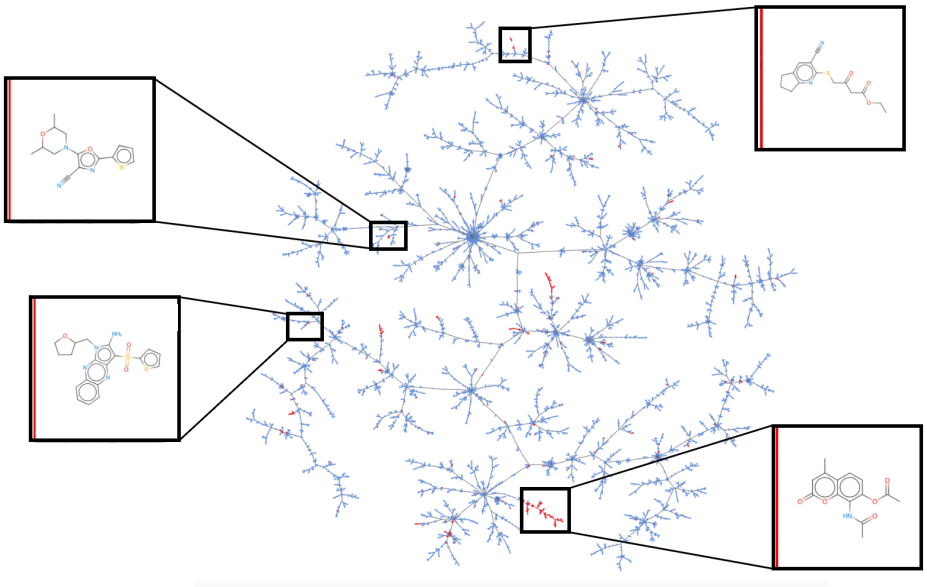
Model performances on the PubChem derived test set



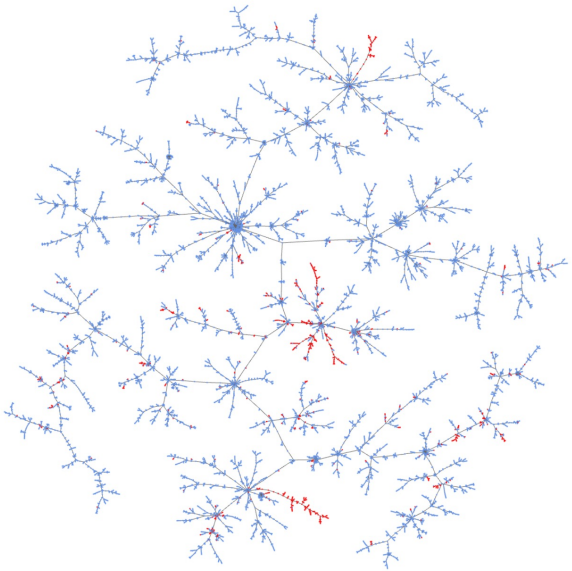
MODEL	MCC	ROC-AUC	Precision	Recall
HitDexter3.0	0.25	0.84	0.18	0.84
ChemFluo	0.34	0.82	0.23	0.75
FI-RF	0.45	0.94	0.34	0.66

universität wien

Analysis of performances on the PubChem derived test set



TMAP of PubChem test set compounds colored by PubChem activity label (BLUE non-autofluorescent, RED autofluorescent)



TMAP of PubChem test set compounds colored by predicted interference label (BLUE non-interfering, RED interfering)

universität wien

Conclusions

- **Single-dose HTS** data can be used with very **little preprocessing** to address assay interference
- We show that statistically derived labels can be used to train ML models for prediction of assay interference (best model reaching **MCC=0.47** on the internal test set)
- The interference labels obtained using ATR, NAR, and Fisher exact test can **approximate experimental evidence**
- Our best model **outperforms existing methods** for the prediction of autofluorescent compounds (**MCC=0.45** on the external test set)

Further experiments

- Explore if the models can predict other type of interference (e.g. aggregation)
- Extend the approach to other assay technologies