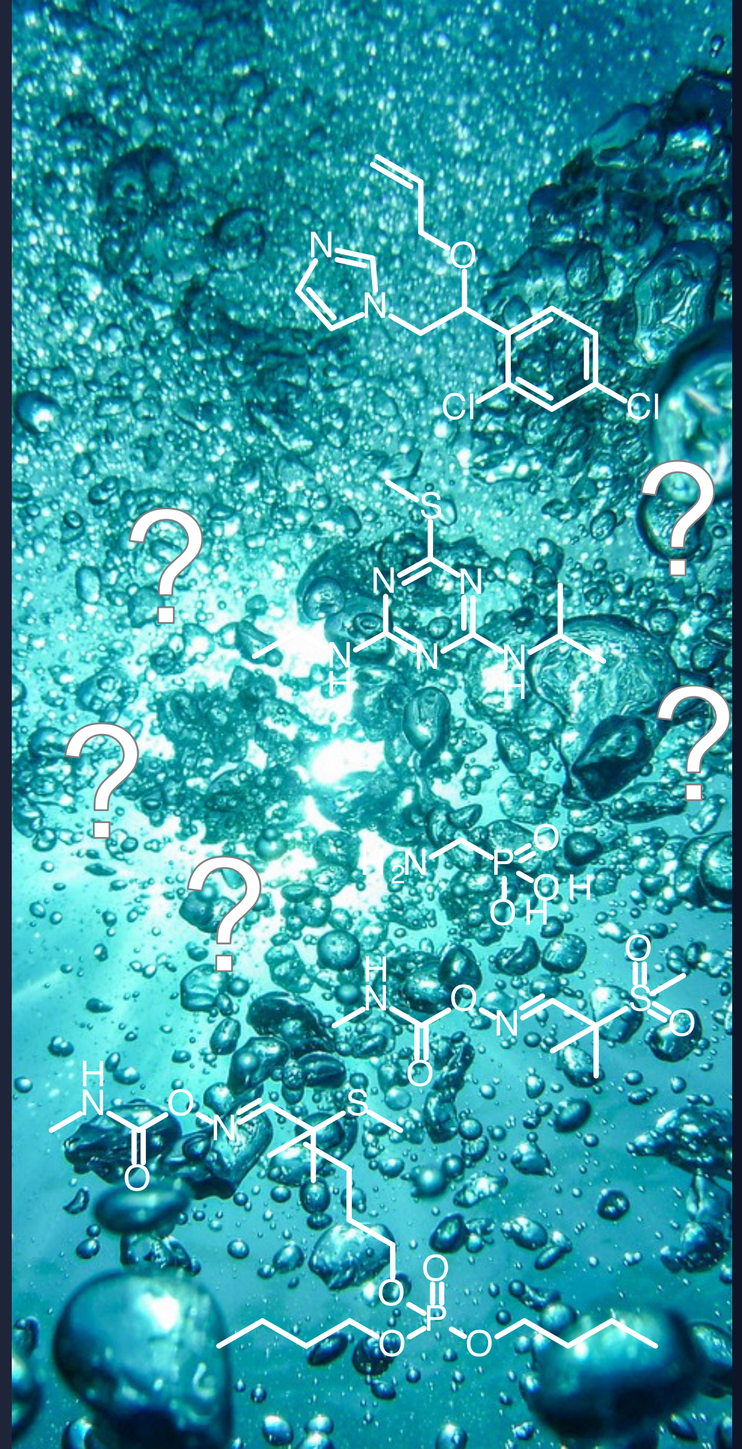
LC/HRMS: prioritizing & elucidating the structure of toxic chemicals

Anneli Kruve

Kruvelab.com

water analysis

thousands of chemicals
detected
in the environment



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how to ...

PRIORITIZE

risk

IDENTIFY

structure

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how to ...

PRIORITIZE

risk

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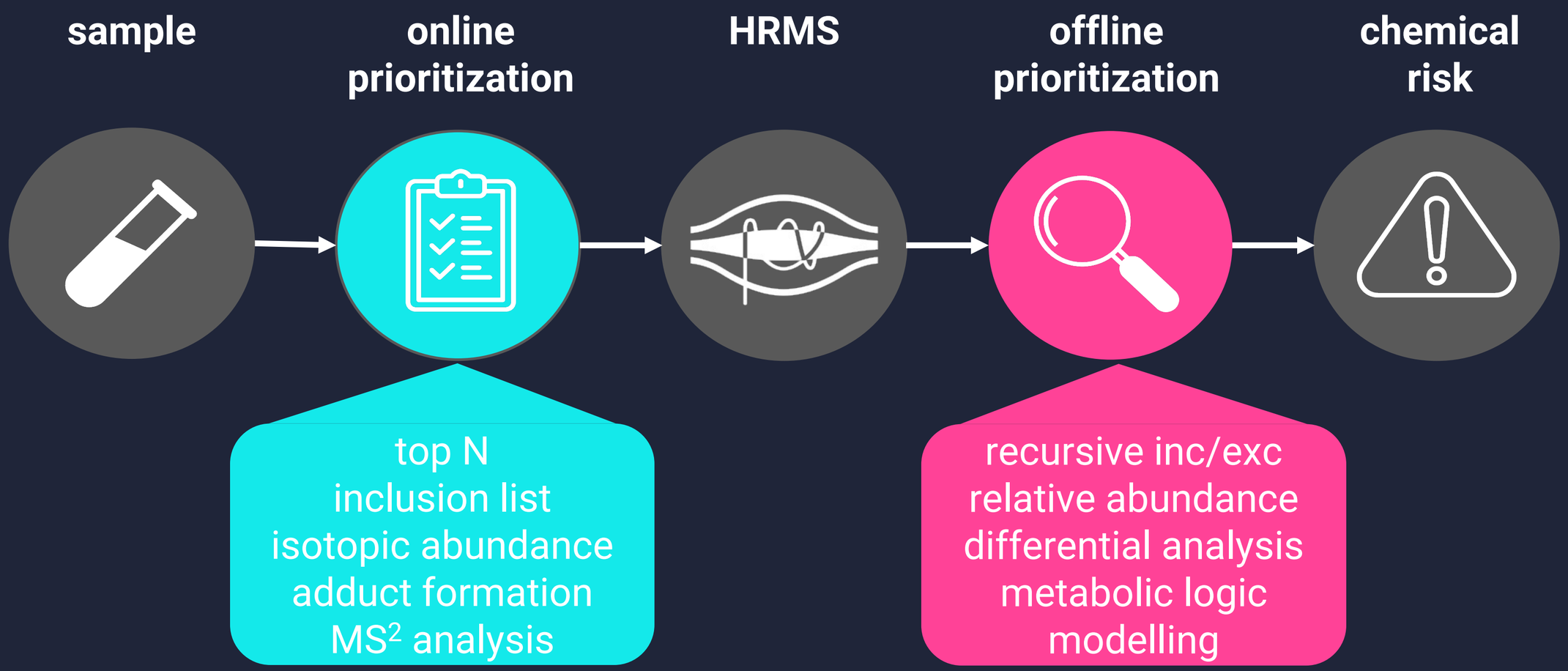
prioritization

Szabo et al. Anal Chem 2024



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prioritization of chemicals

TOXICITY

ecotoxicity and endocrine disruptors

CONCENTRATION

exposure to potentially toxic chemicals

RISK

$$\text{PriorityScore} = \frac{\text{C}_{\text{predicted}}}{\text{AC}_{50}^{\text{5th percentile}}}$$

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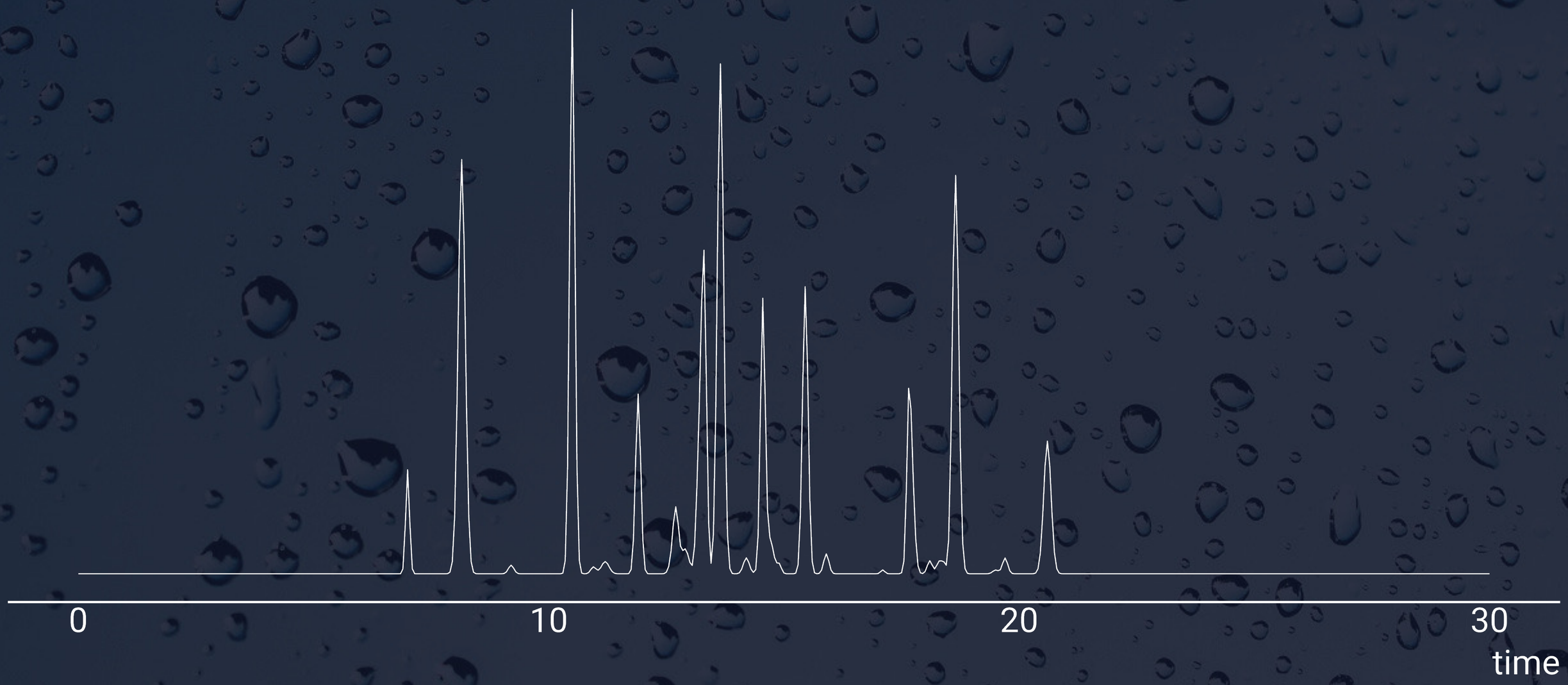
toxicity

of detected chemicals

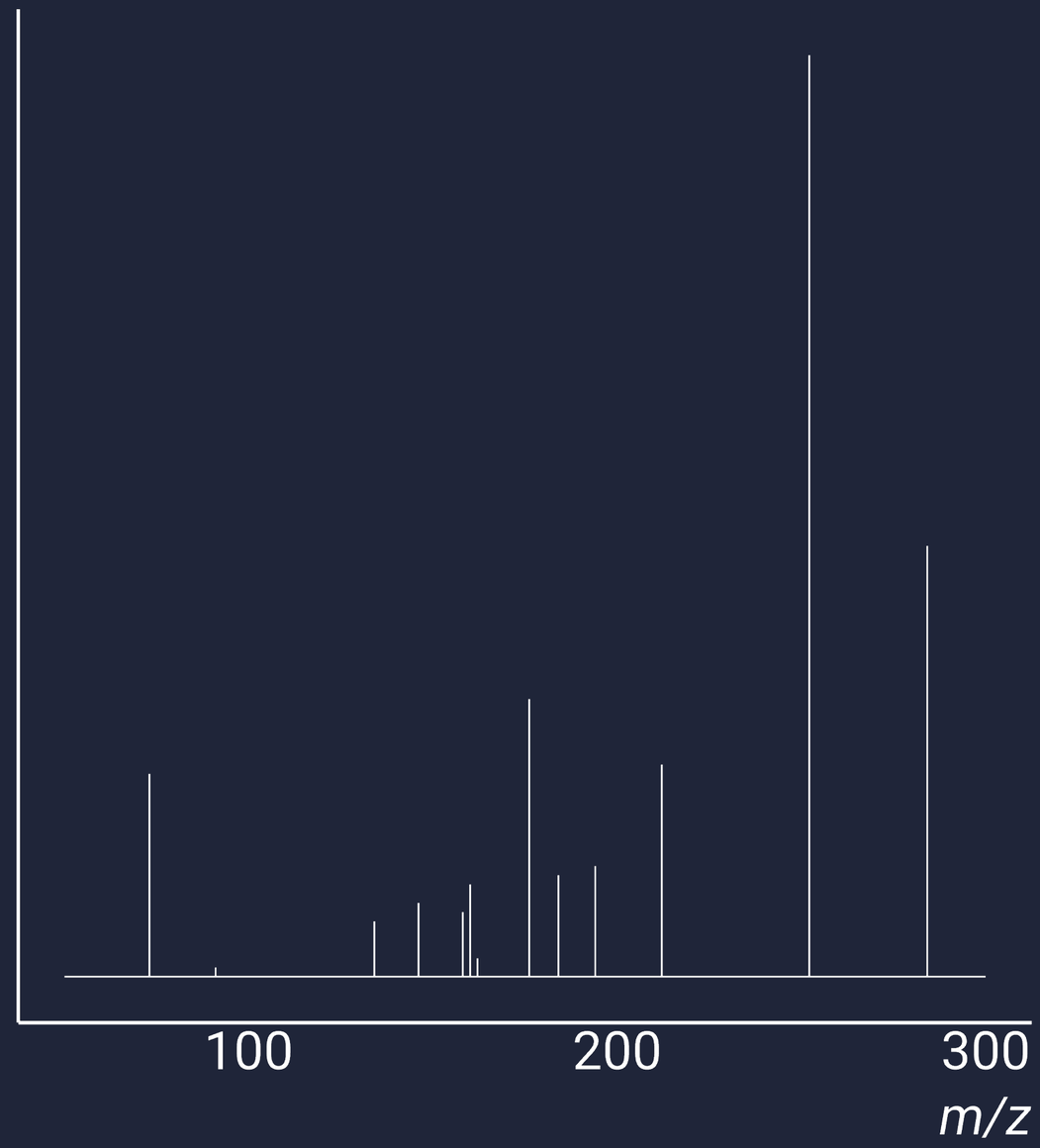
nontarget screening

with LC/HRMS



toxicity assessment

from spectra
to structure
to toxicity



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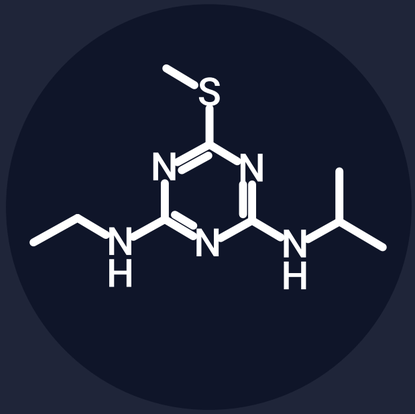
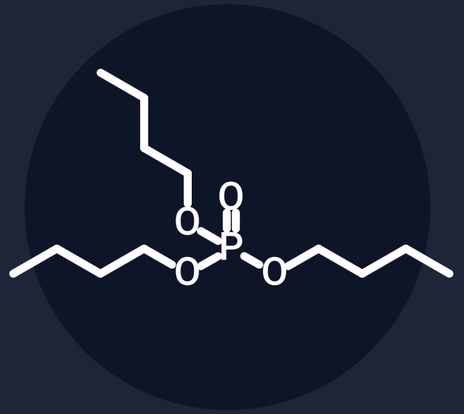
toxicity assessment



LC_{50} = 9.3 mg/L

known structure
known toxicity



LC_{50} = ? mg/L

known structure
unknown toxicity

LC_{50} = ? mg/L

unknown structure
unknown toxicity

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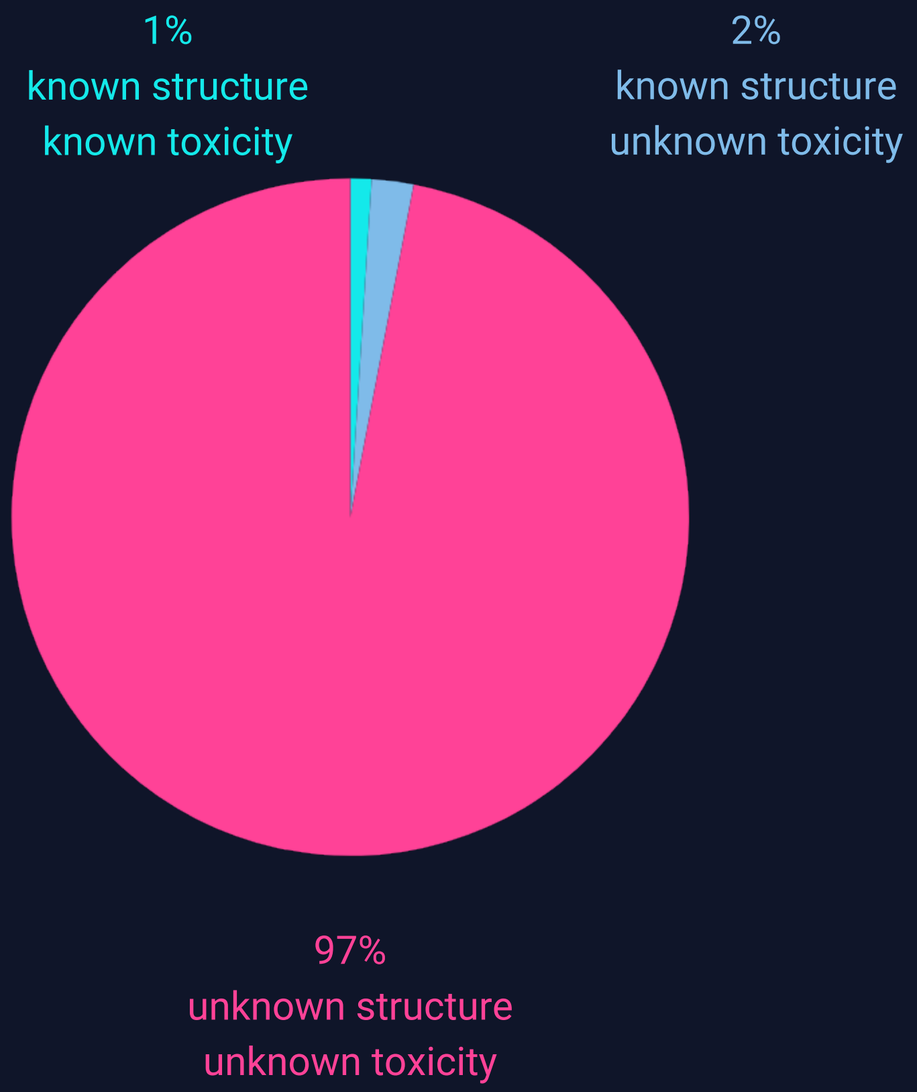

toxicity assessment

vast majority of detected chemicals remain unknown

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predicting toxicity

of detected chemicals

endpoints

ECOTOXICITY

fathead minnow, bluegill, and rainbow trout

ENDOCRINE DISRUPTION

AhR, AR, ER, MMP, P53, ...

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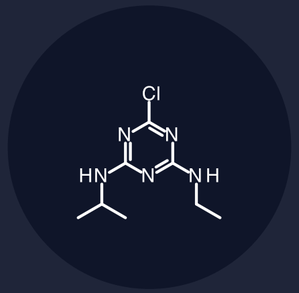
workflow

MS^2 SPECTRA



STRUCTURE AS SMILES

MOLECULAR DESCRIPTORS

PREDICT TOXICITY

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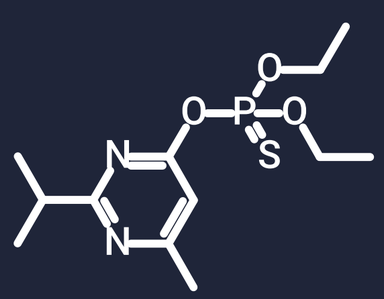
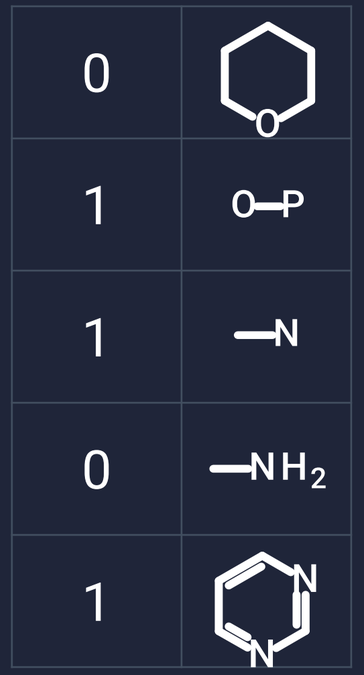
structural fingerprints

Peets et al. ES&T 2022



R:
rcdk



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model training

Peets et al. ES&T 2022

mass (Da)	fp1	...	fp243
317.32000	0	...	0
208.26100	1	...	0
240.21499	1	...	0
300.57998	0	...	0
201.22500	0	...	0

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model training

Peets et al. ES&T 2022

mass (Da)	fp1	...	fp243
317.32000	0	...	0
208.26100	1	...	0
240.21499	1	...	0
300.57998	0	...	0
201.22500	0	...	0

training set
517
chemicals

test set
130
chemicals

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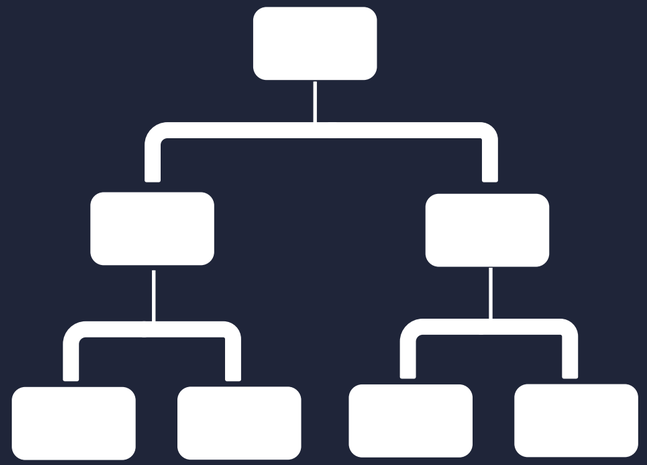
model training

Peets et al. ES&T 2022

mass (Da)	fp1	...	fp243
317.32000	0	...	0
208.26100	1	...	0
240.21499	1	...	0
300.57998	0	...	0
201.22500	0	...	0

gradient boosting



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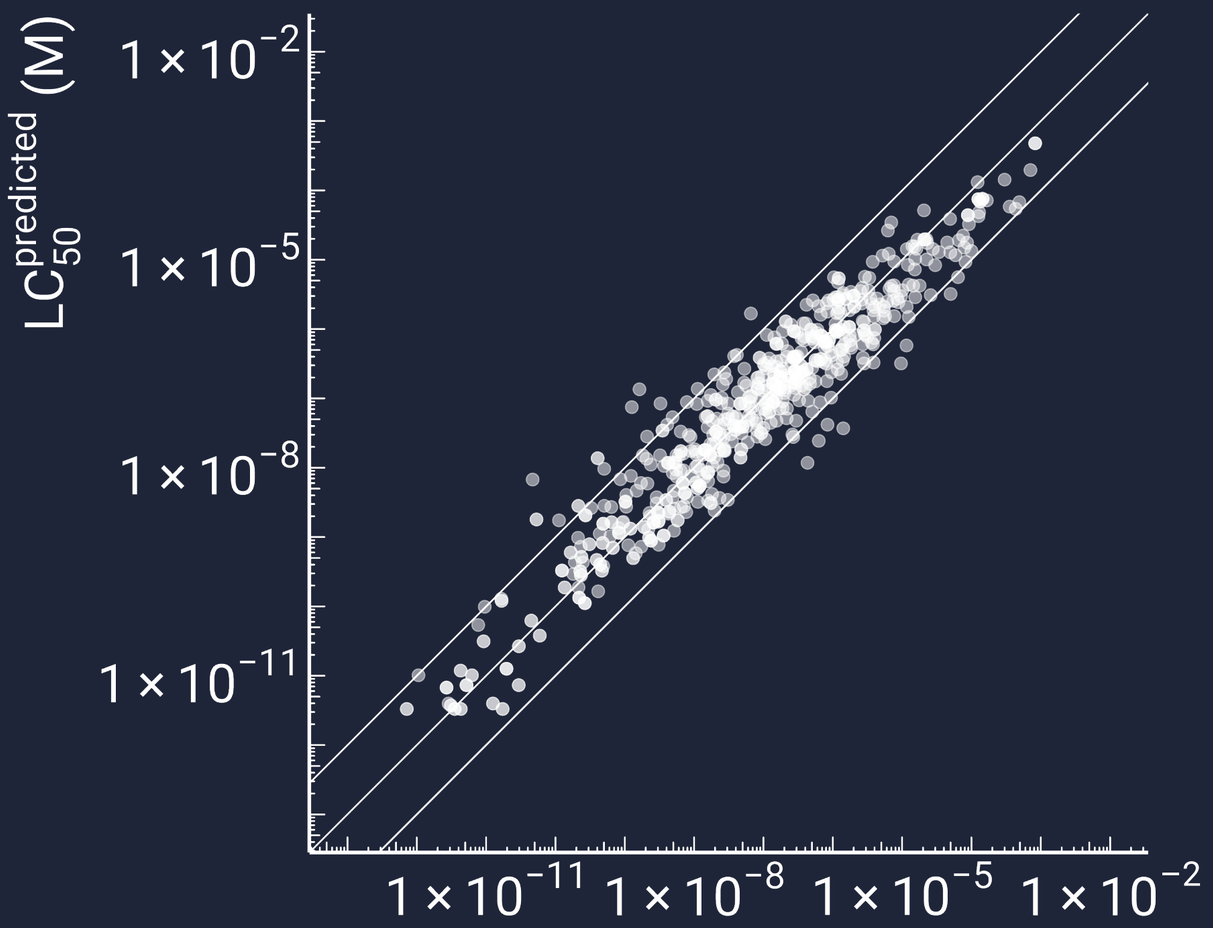
LC_{50} predictions

Peets et al. ES&T 2022
fish LC_{50}



training set

RMSE 0.52 log(M)

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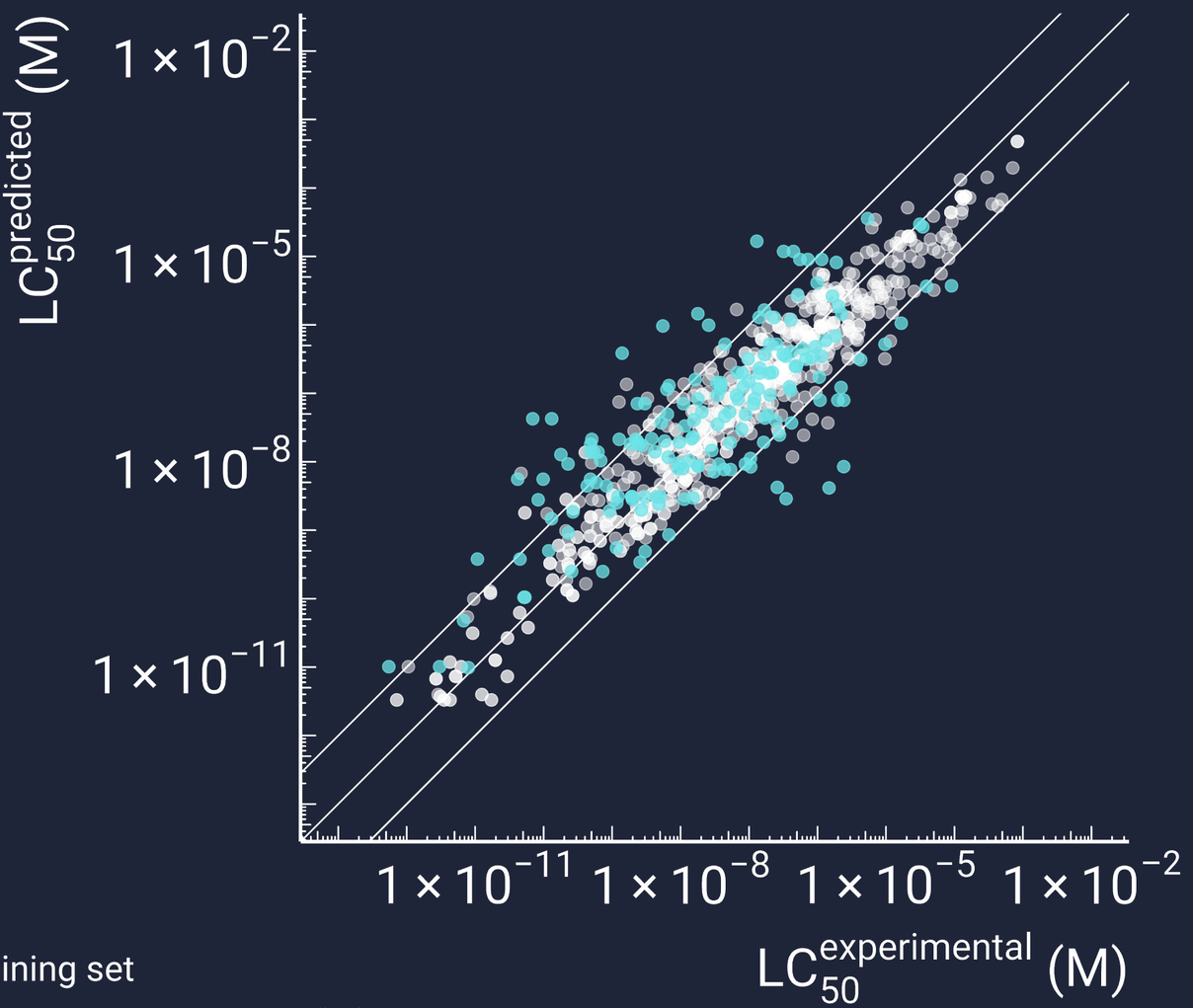
LC_{50} predictions

Peets et al. ES&T 2022

fish LC_{50}



training set

RMSE 0.52 log(M)

test set

RMSE 0.78 log(M)

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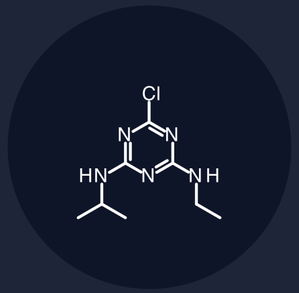
predicting toxicity

of unidentified chemicals

workflow

MS^2 SPECTRA



STRUCTURE AS SMILES

MOLECULAR DESCRIPTORS

PREDICT TOXICITY

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workflow

MS^2 SPECTRA

MOLECULAR DESCRIPTORS

PREDICT TOXICITY

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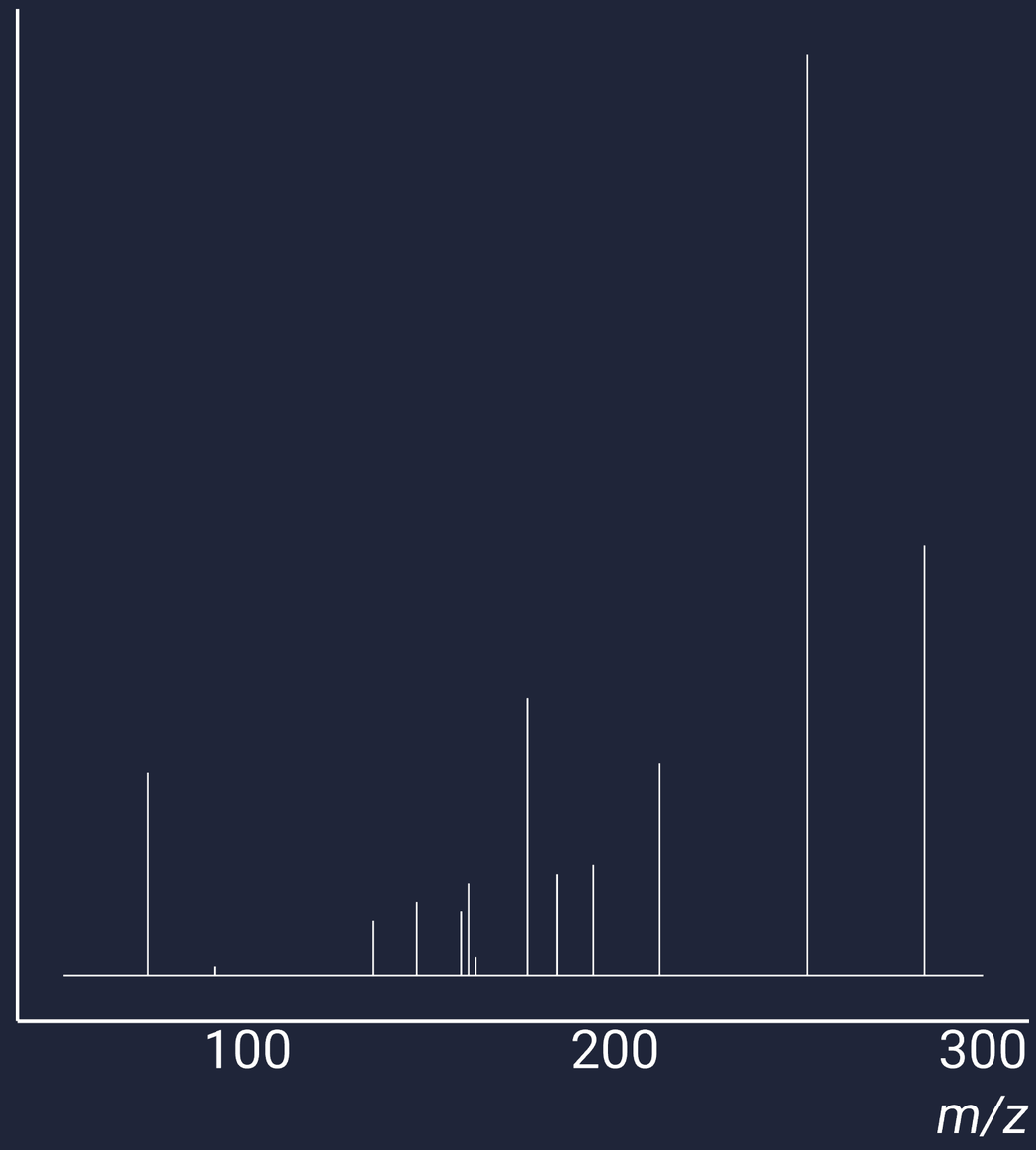
information available

in MS^2 spectra



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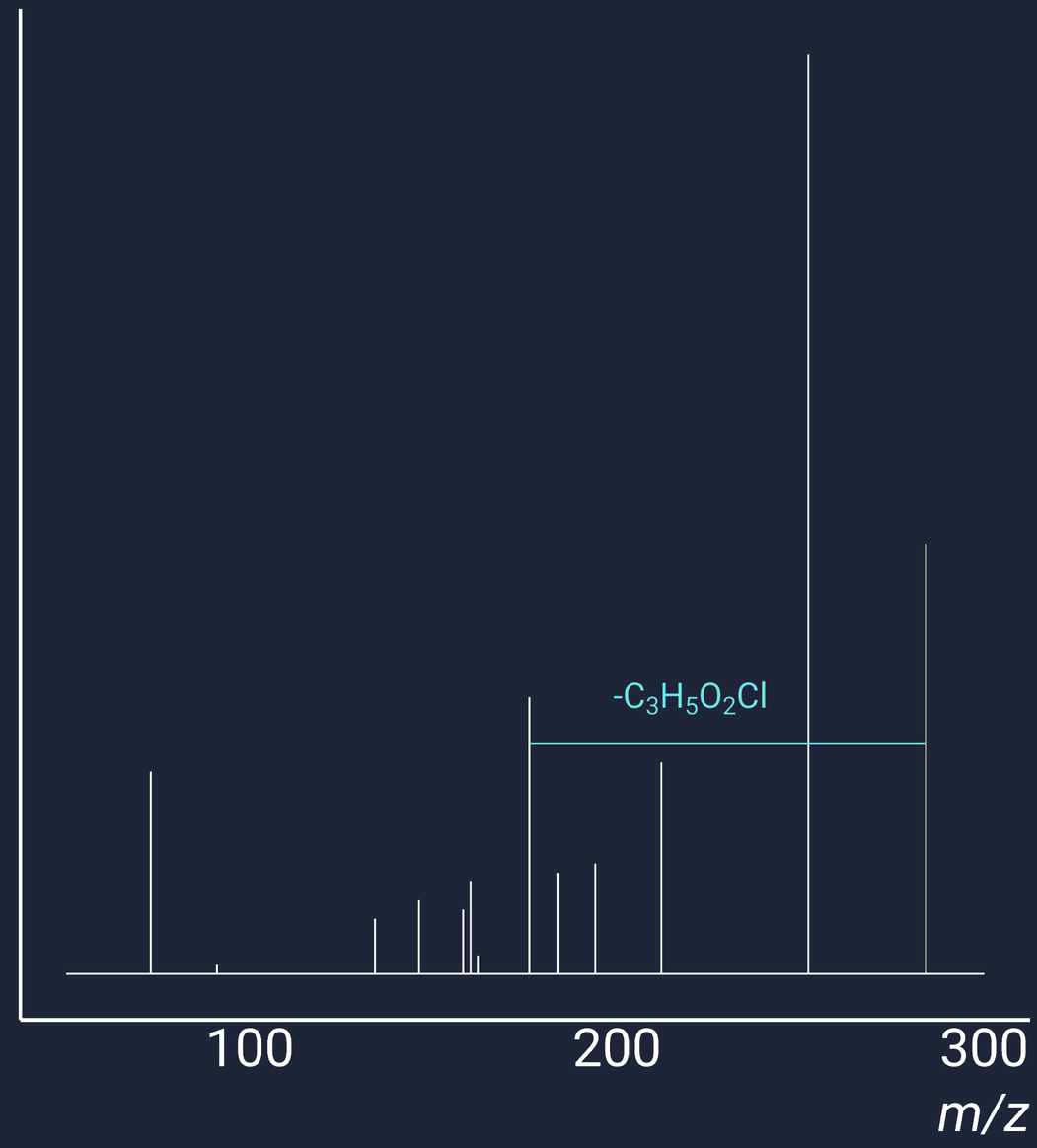
information available

in MS^2 spectra



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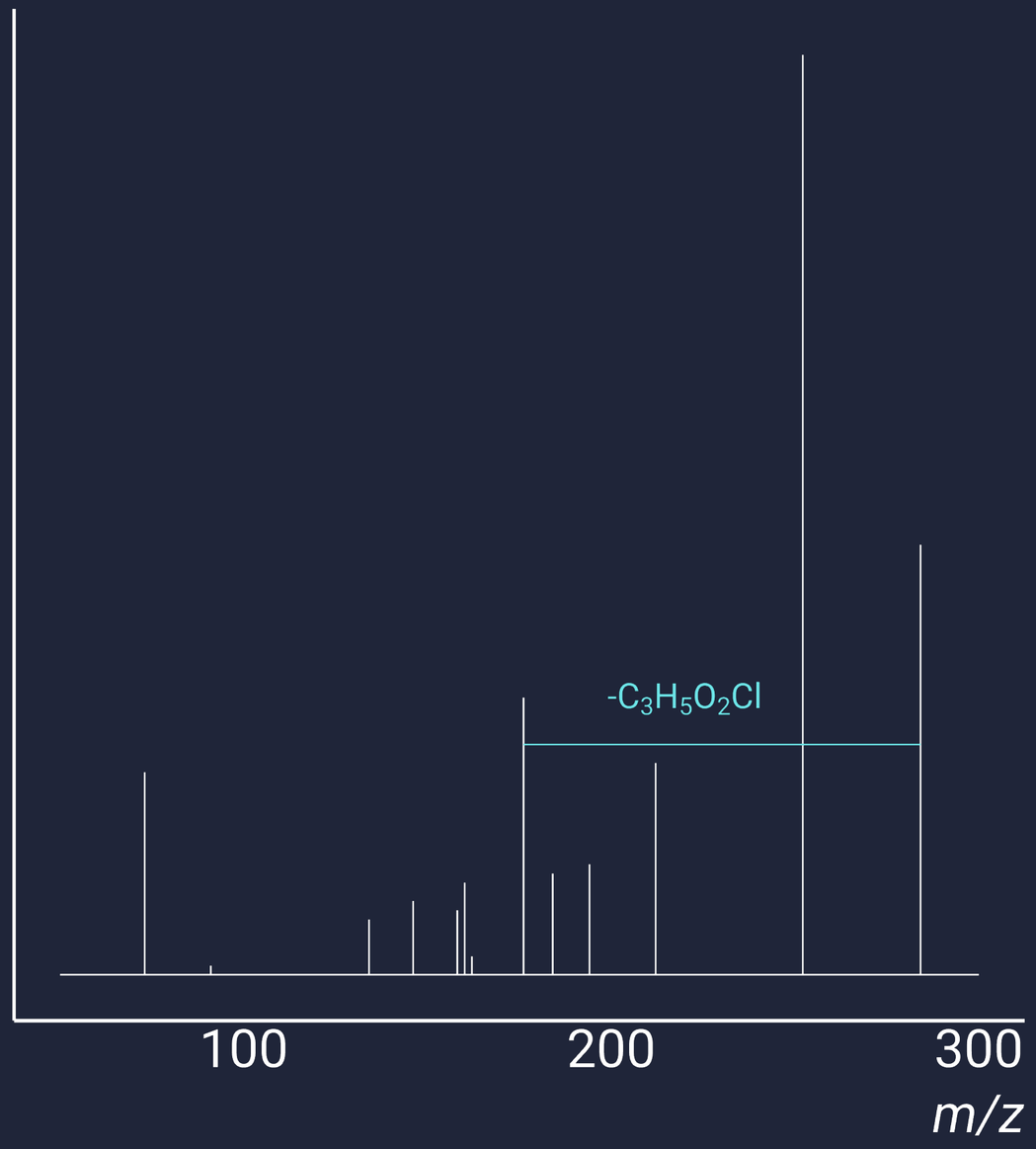

predict toxicity

for unknown chemicals

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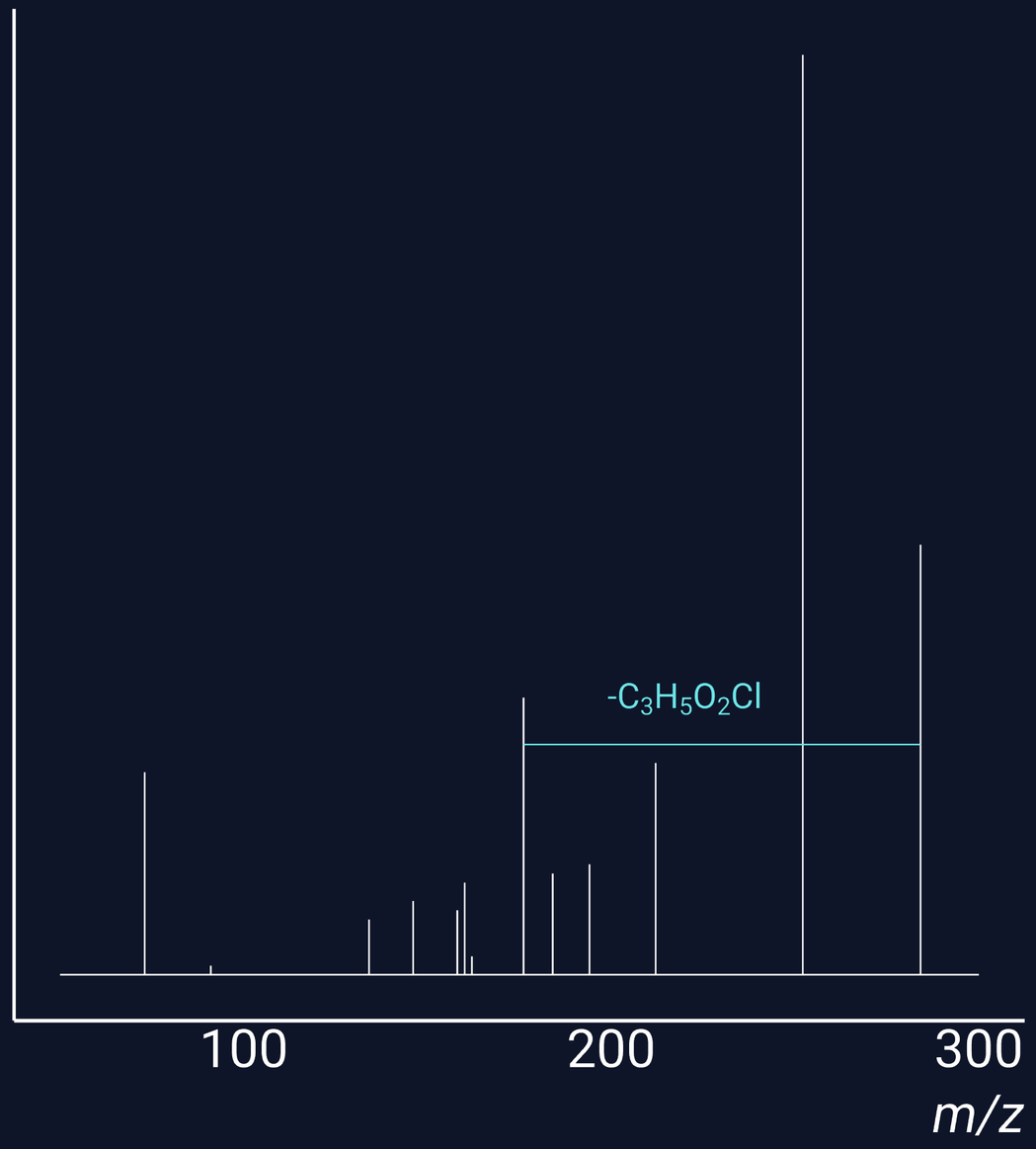
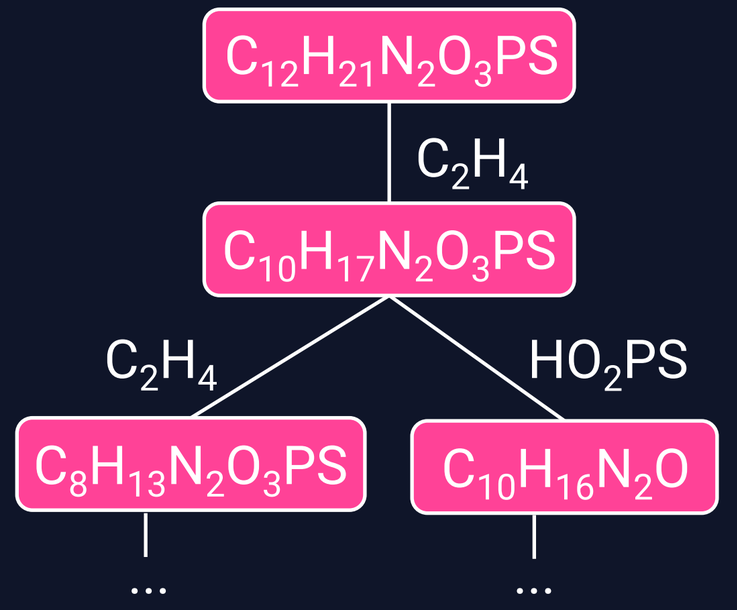


SIRIUS+
CSI:FingerID



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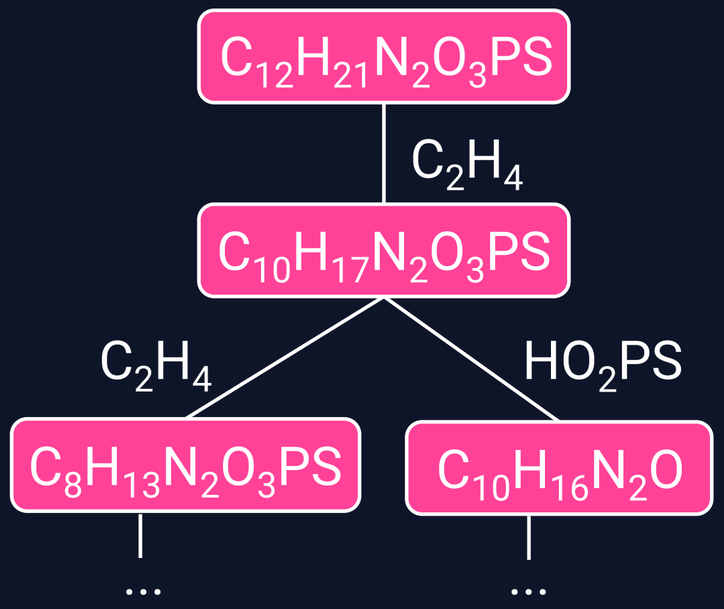

SIRIUS+
CSI:FingerID

0	
1	
1	
0	
1	

gradient
boosting

LC_{50} =
−2.2 log(mM)

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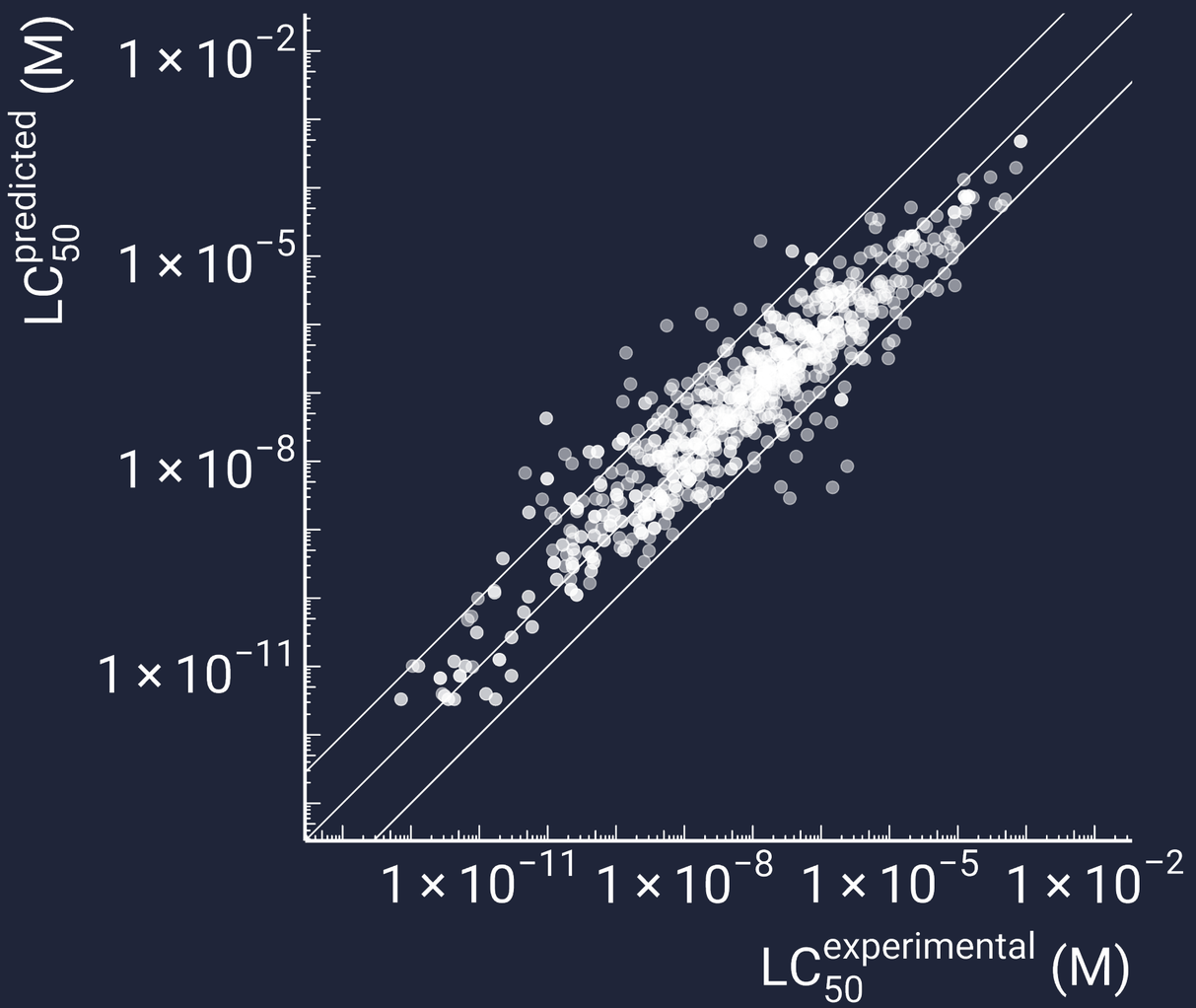
LC_{50} predictions

Peets et al. ES&T 2022

fish LC_{50}



test set

RMSE 0.78 log(M)

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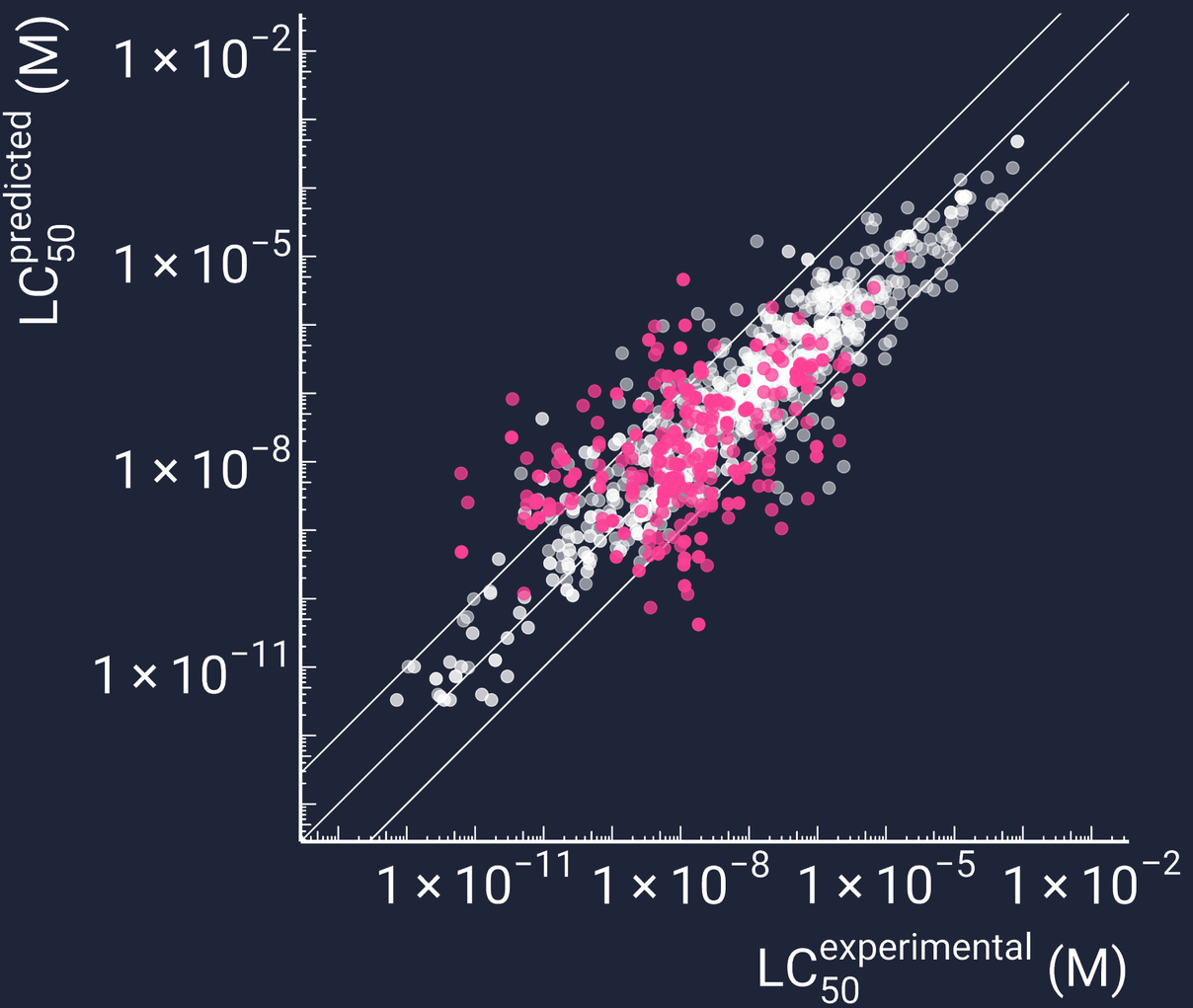
LC_{50} predictions

Peets et al. ES&T 2022

fish LC_{50}



validation on MassBank

$RMSE_{model}$ 0.88 log(M)

$SD_{experimental}$ 0.44 log(M)

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endocrine disruption

Rahu et al. ES&T 2024
Tox21 endpoints

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endocrine disruption

Rahu et al. ES&T 2024
Tox21 endpoints

true label	
active	non-active

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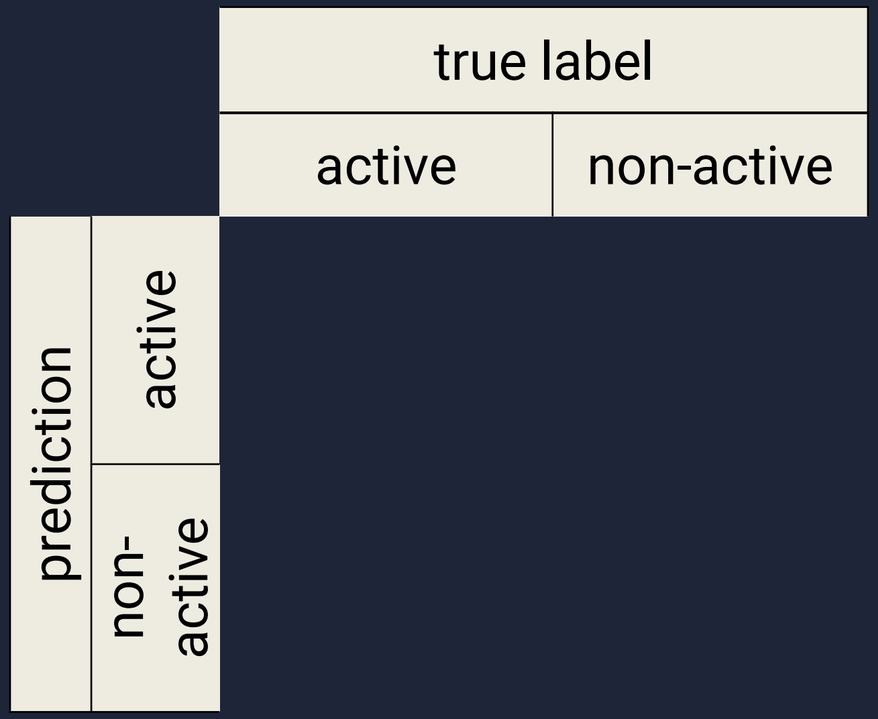
endocrine disruption

Rahu et al. ES&T 2024
Tox21 endpoints



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endocrine disruption

Rahu et al. ES&T 2024
Tox21 endpoints

		true label	
		active	non-active
prediction	active	TP	FP
	non-active	FN	TN

which is more dramatic:

type I error
type II error

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endocrine disruption

Rahu et al. ES&T 2024
Tox21 endpoints

		true label	
		active	non-active
prediction	active	TP	FP
	non-active	FN	TN

FPR @ TPR = 0.9

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endocrine disruption

Rahu et al. ES&T 2024
Tox21 endpoints

bioassay	FPR
sr.mmp	25.1%
sr.p53	25.4%
nr.ahr	41.8%
…	…
nr.ar	82.4%
nr.er	85.0%

MassBank & MoNA

748 compounds with MS^2 & tox

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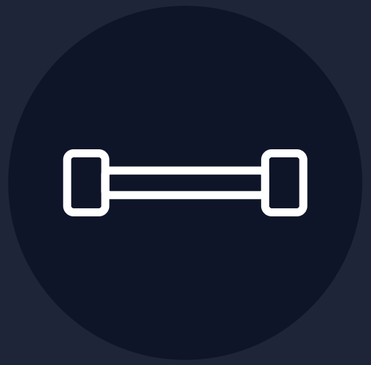
case study: interlaboratory comparison

interlaboratory comparison

Sandberg, Rahu, in preparation

SAMPLES

spiked water samples



ANALYSIS

HRMS, etc. characterization

DATA PROCESSING

AhR activity

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results

4700

LC/HRMS features detected

238

features with MS^2 spectra

55

features predicted active

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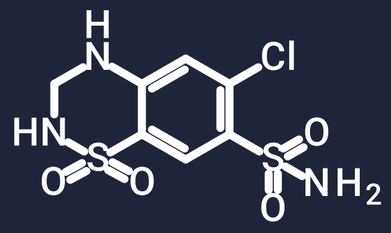
AhR active

hydrochlorothiazide



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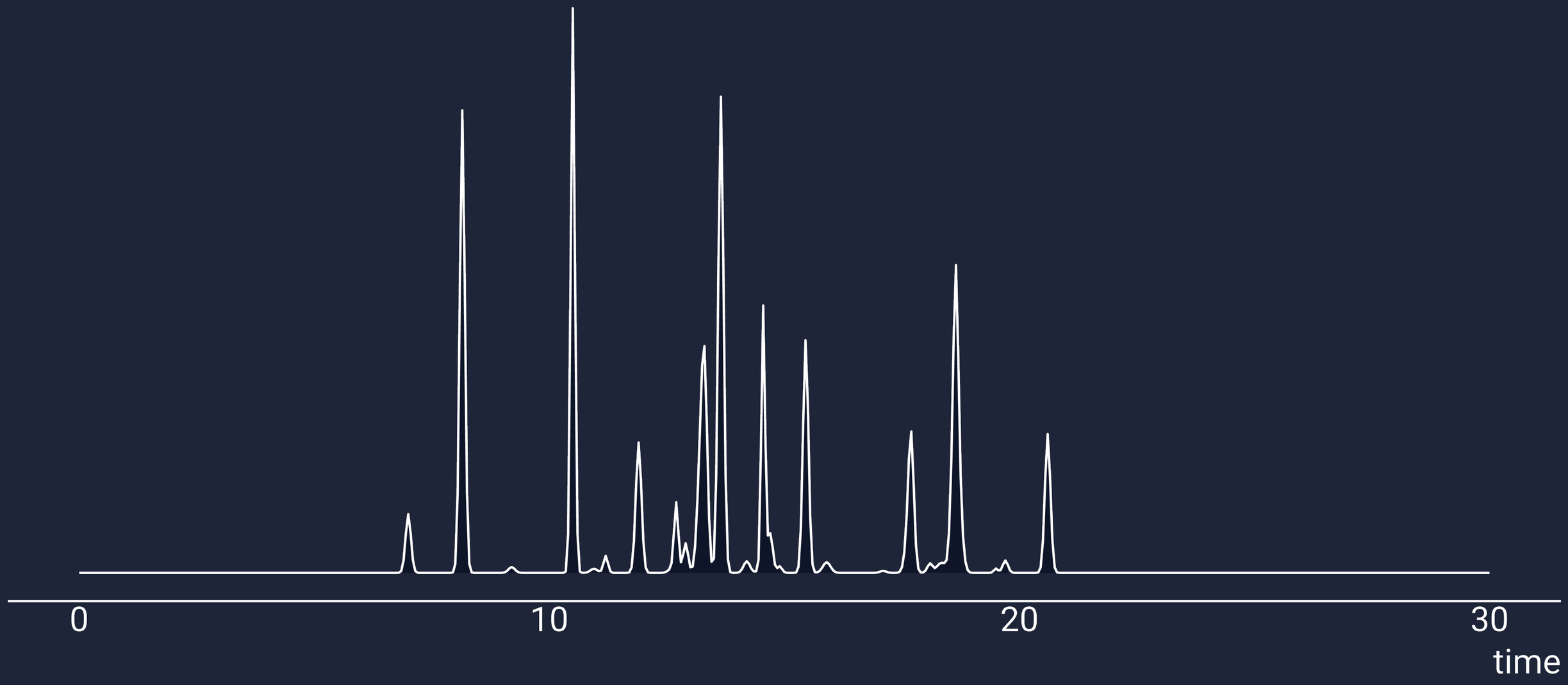
quantification

of detected chemicals

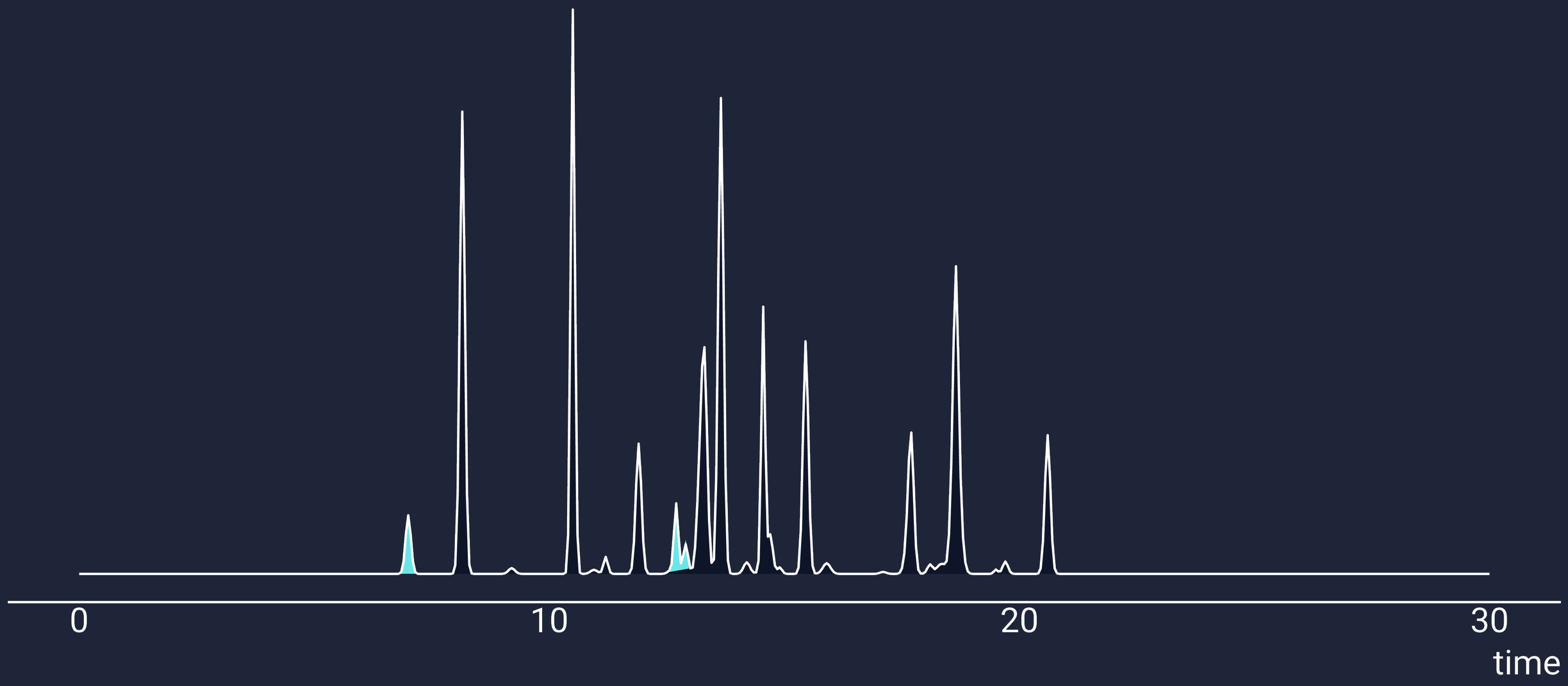
quantification in ESI/HRMS

Malm et al. Molecules 2021



quantification in ESI/HRMS

Malm et al. Molecules 2021



quantification in ESI/HRMS

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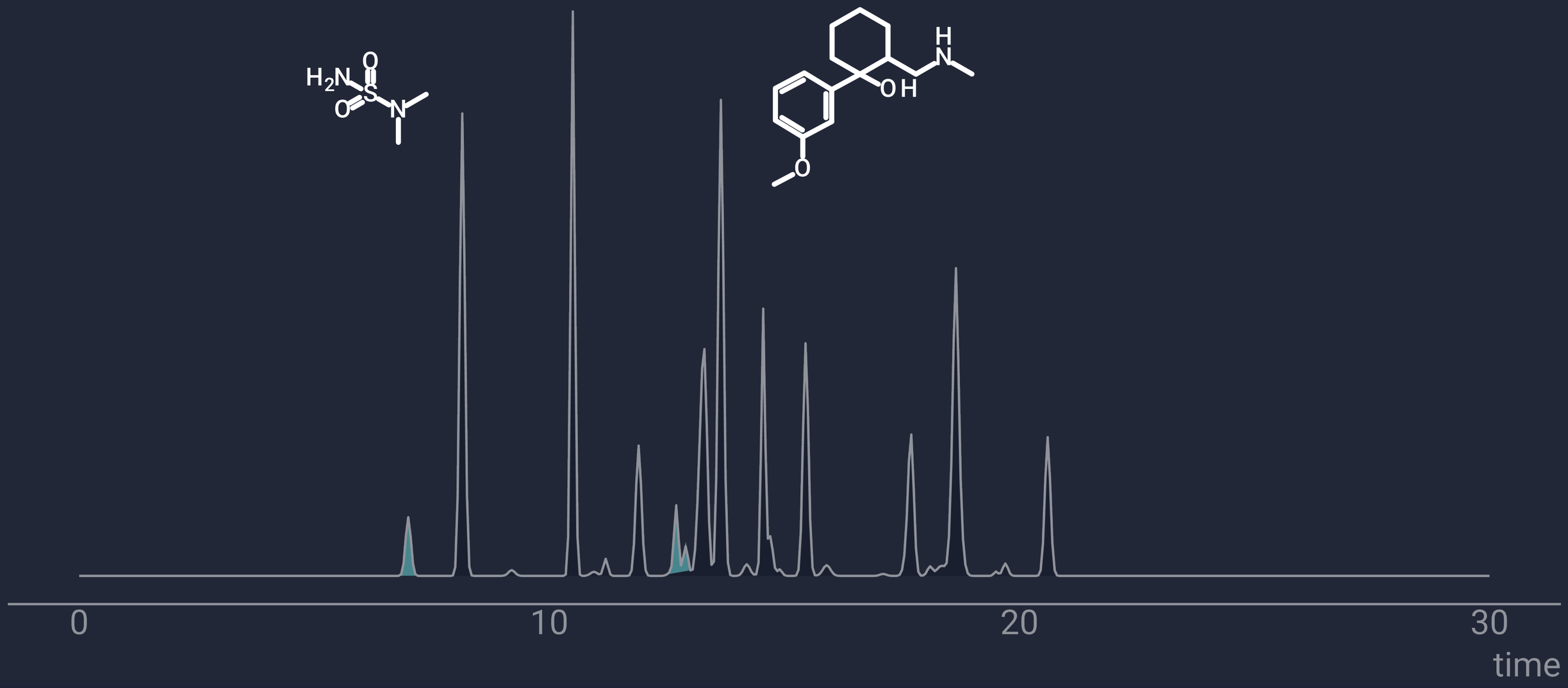
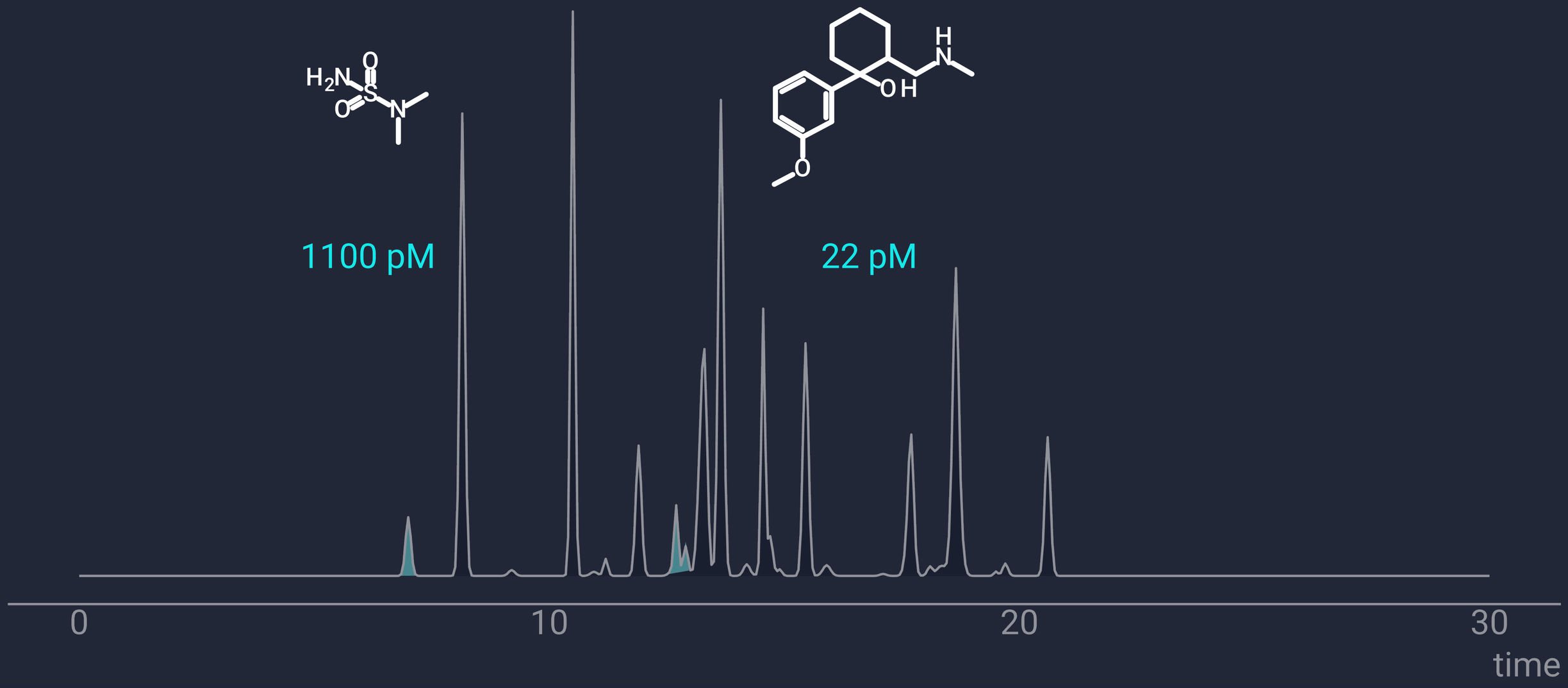
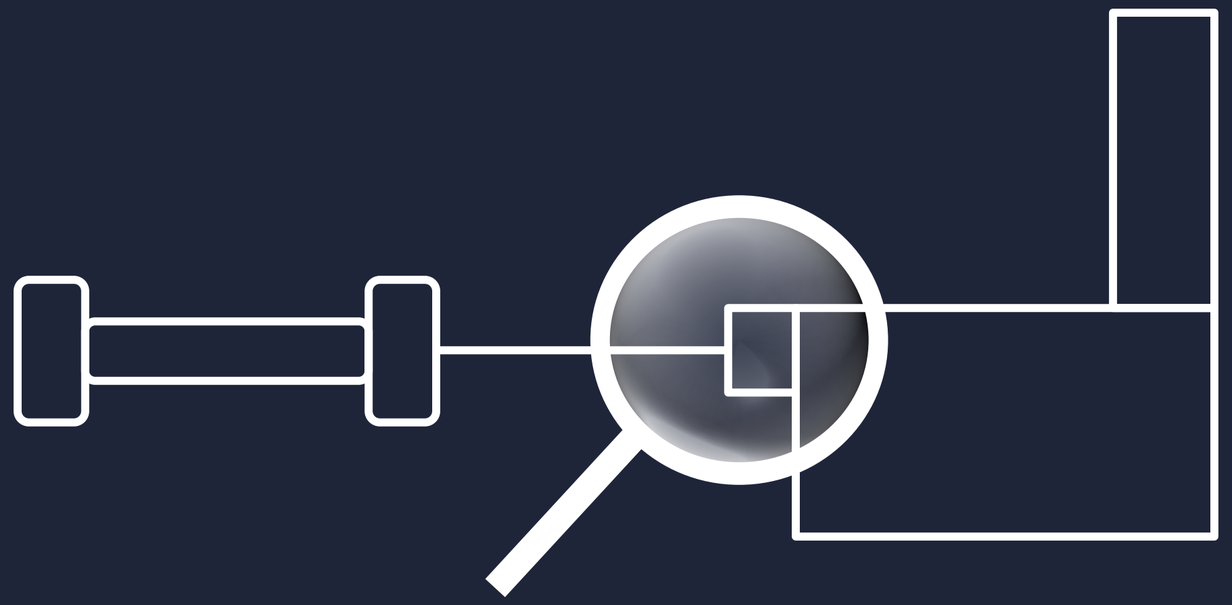


quantification in ESI/HRMS

Malm et al. Molecules 2021



electrospray



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ionization efficiency

ANALYSIS

flow injections

DATA

calibration graphs

$$\frac{slope_1}{slope_2} \rightarrow IE$$

IONIZATION EFFICIENCY

relative measurements

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ionization efficiency

one solvent, purely analyte properties
377 chemicals
10,000,000x difference in *IE*

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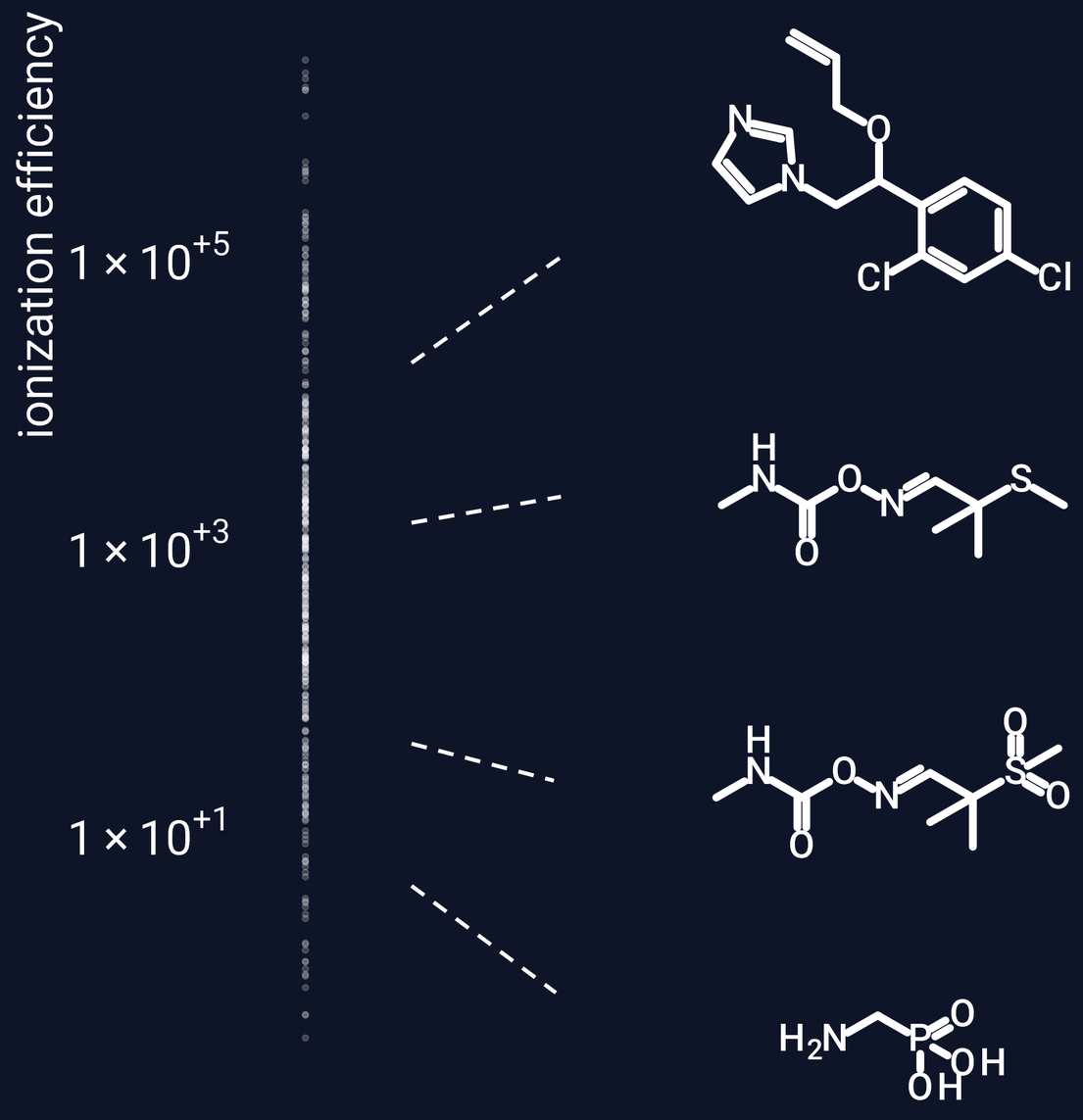

ionization efficiency

one solvent, purely analyte properties
377 chemicals
10,000,000x difference in *IE*

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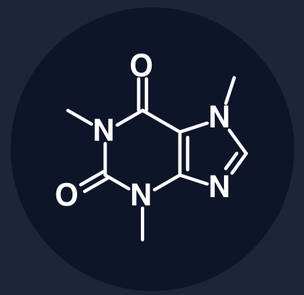
ionization efficiency



STRUCTURE

MOBILE PHASE

INSTRUMENT

MATRIX

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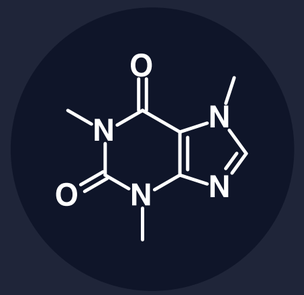
quantification

with machine learning

workflow



SMILES or MS^2

+ mobile phase composition

MOLECULAR DESCRIPTORS

PaDEL, Mordred, SIRIUS fingerprints

MODEL TRAINING

RandomForest, xgbTree

MODEL SELECTION

Root Mean Square Error (RMSE)

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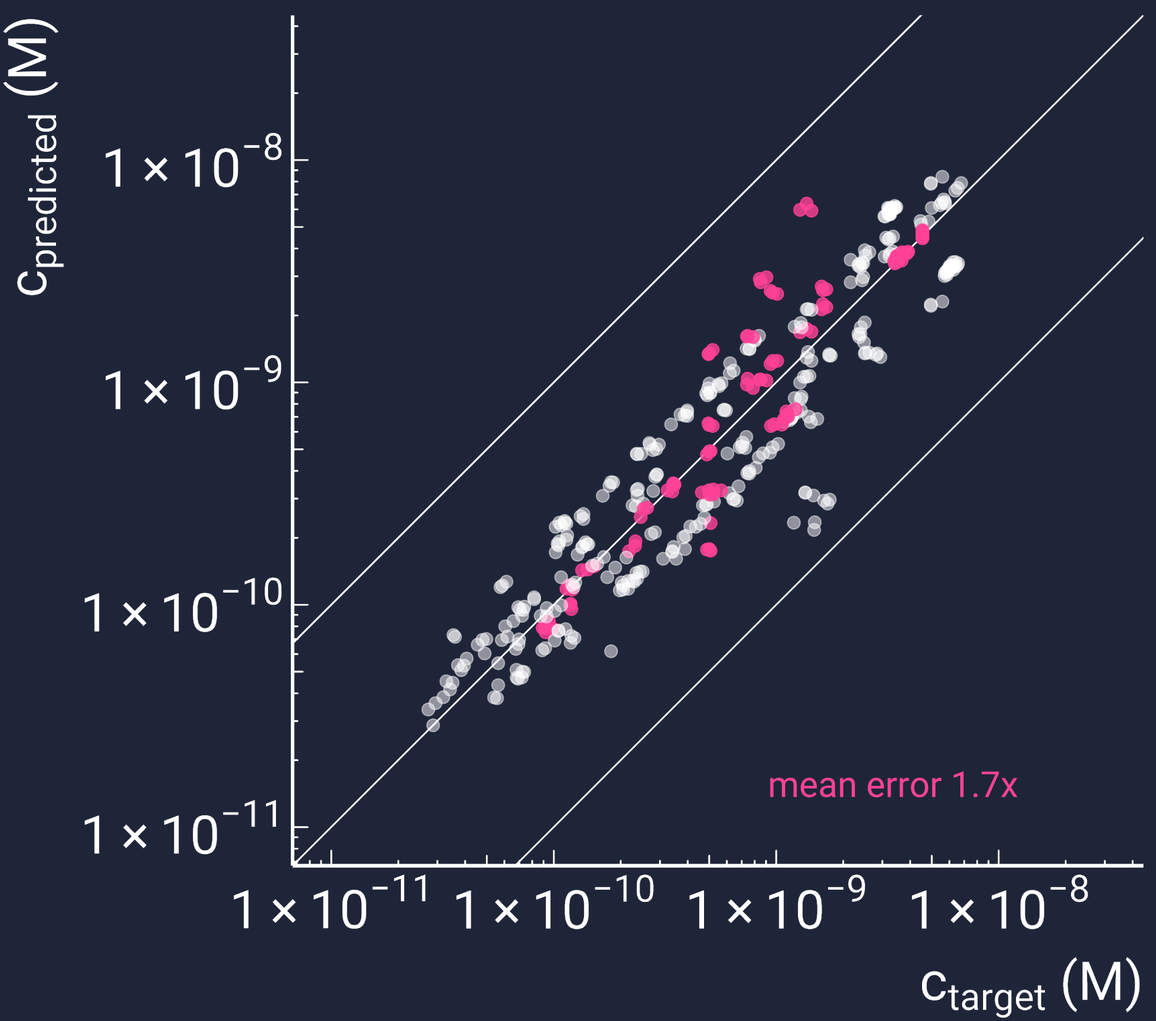
quantification

from structure

Been et al. Water Research 2021



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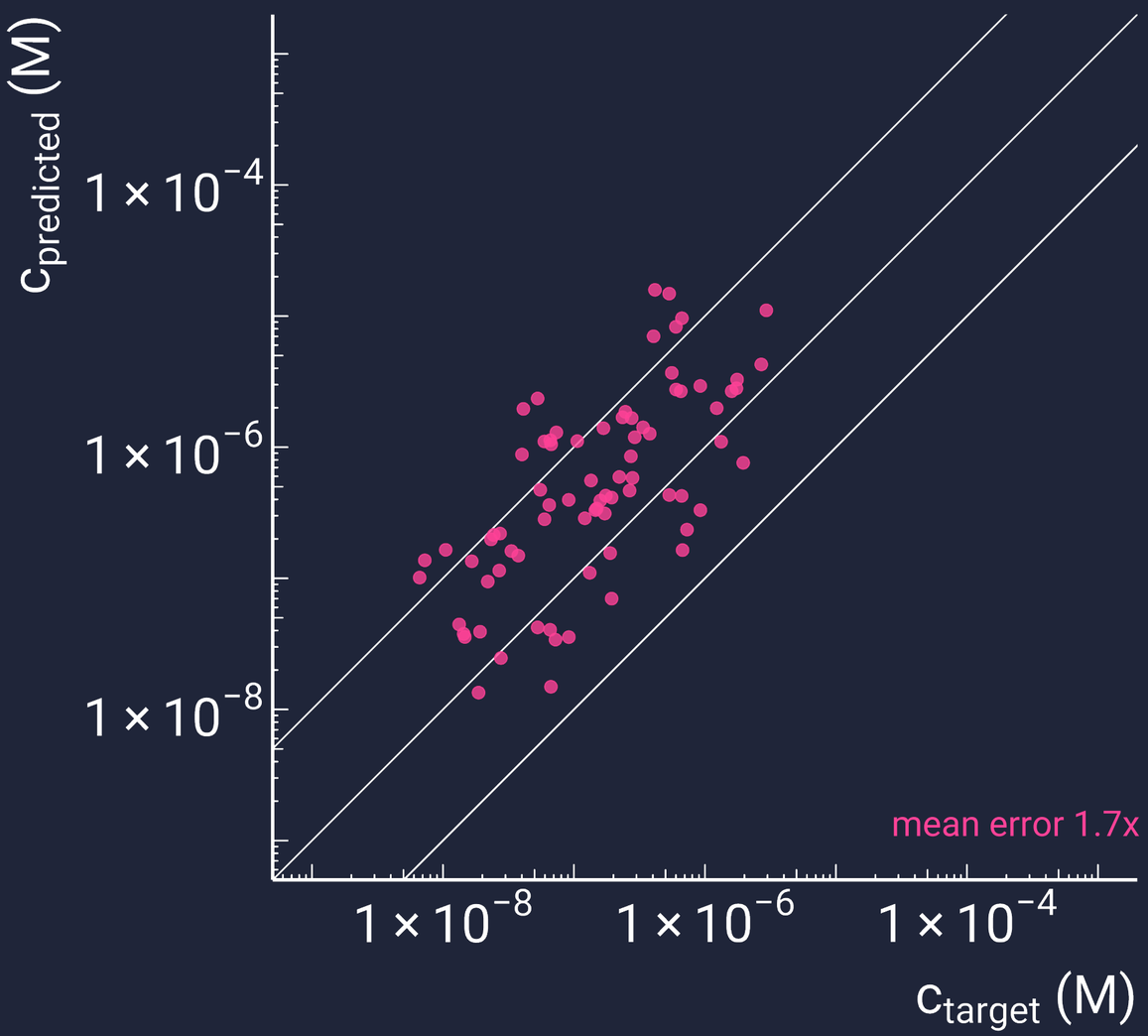
quantification

from MS^2 spectra

Sepman et al. Anal Chem 2023



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how to ...

PRIORITIZE

risk

IDENTIFY

structure

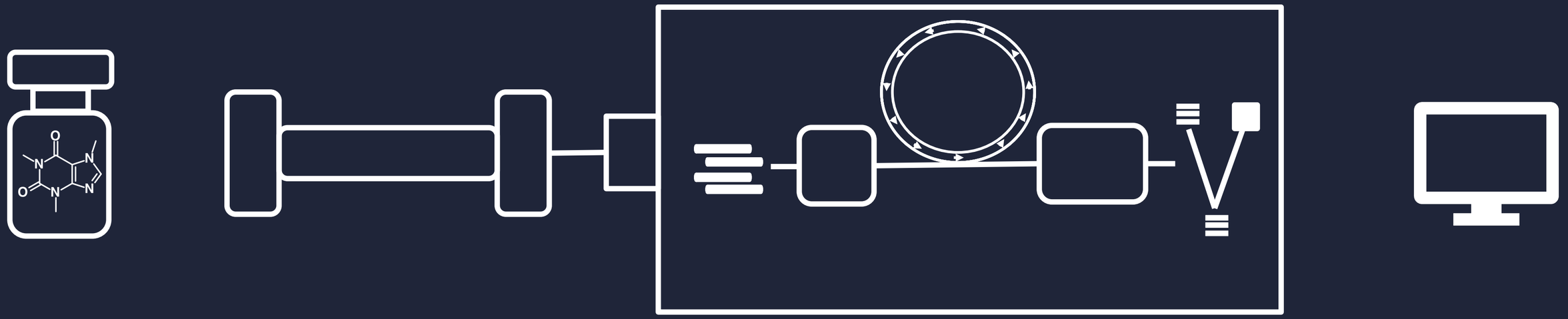
how to ...

IDENTIFY

structure

orthogonal separation

Akhlaqi et al. Anal Bioanal Chem 2023



14
isomeric
chemicals

C_{18} RP

Cyclic IMS
&
MS^2 with ToF

SIRIUS+
CSI:FingerID
&
CFM-ID

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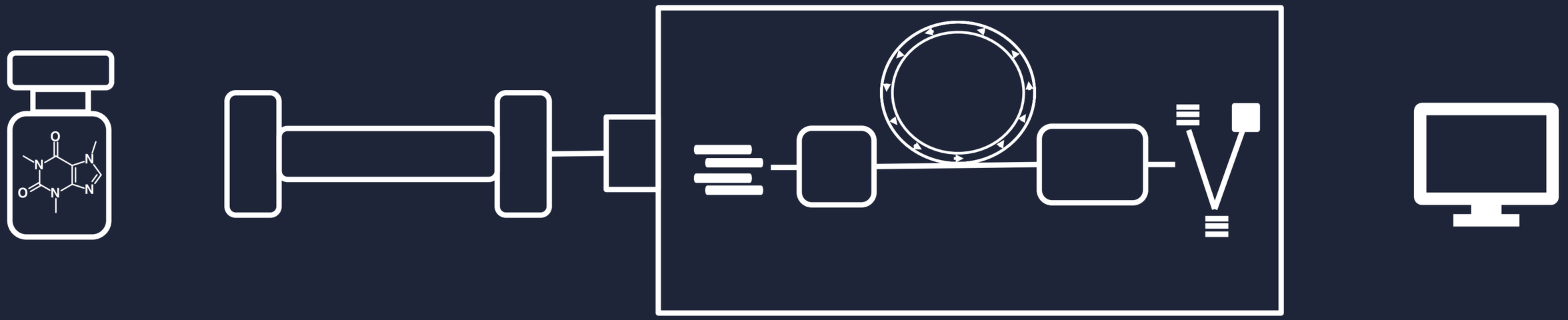
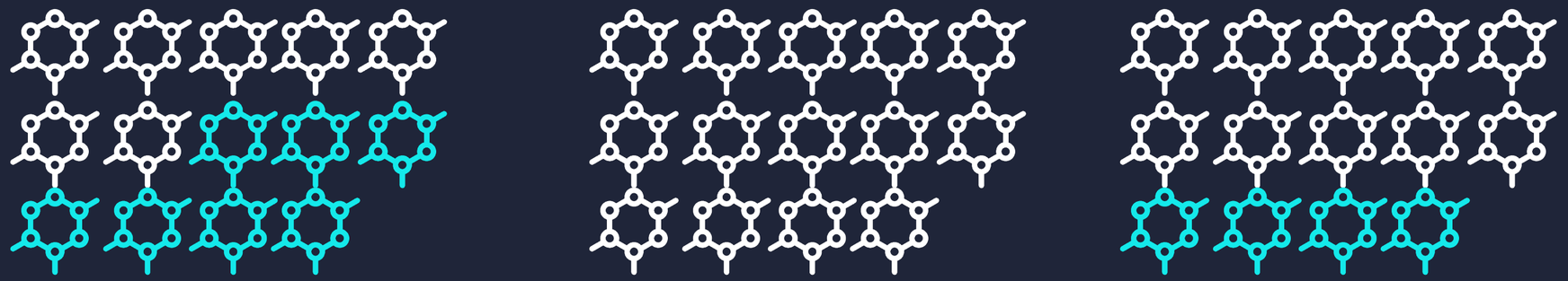
orthogonal separation

Akhlaqi et al. Anal Bioanal Chem 2023



t_R *CCS* MS^2



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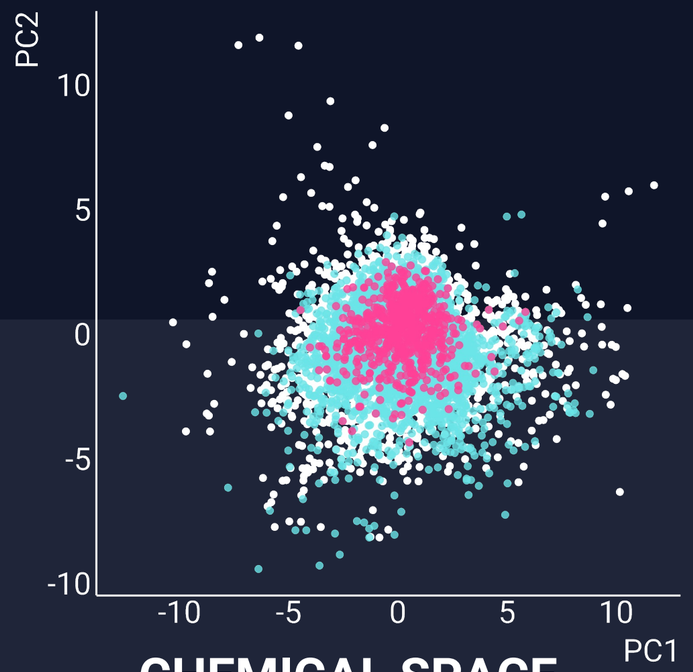
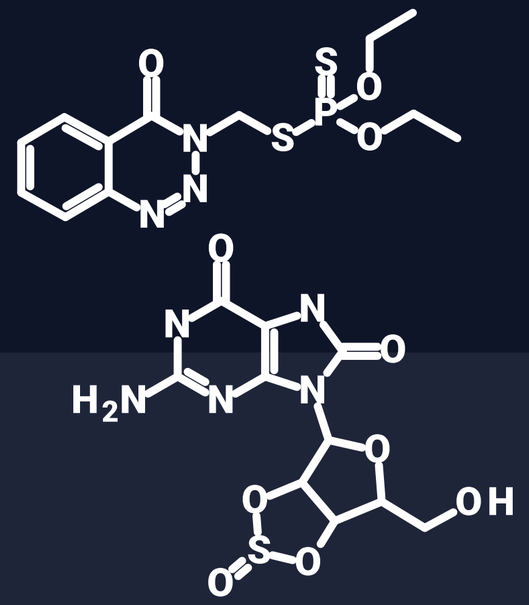
where is the problem



CHEMICAL SPACE

low chemical space coverage



CANDIDATE STRUCTURES

ability to distinguish isomers

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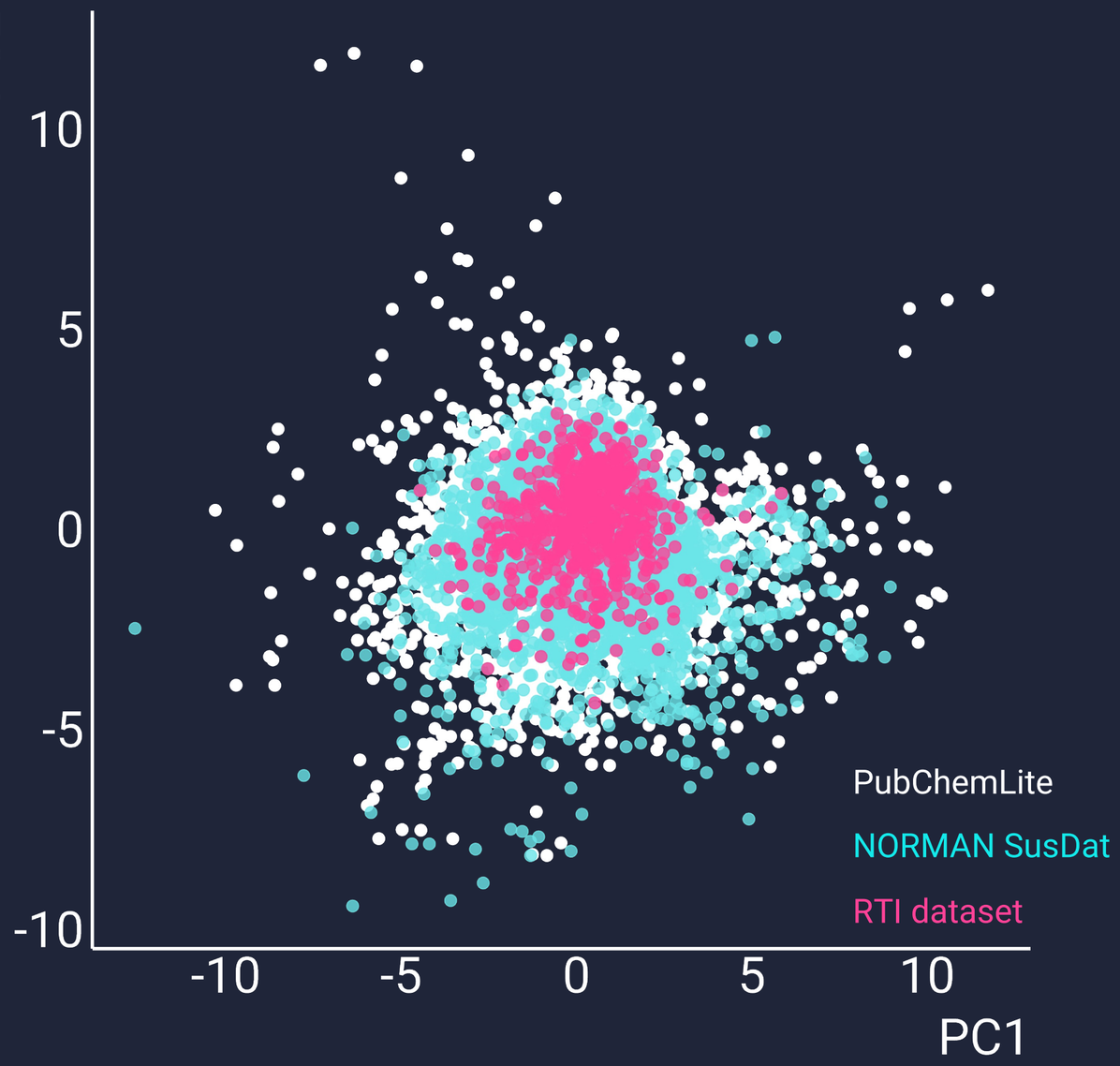
chemical space

covered by prediction algorithms



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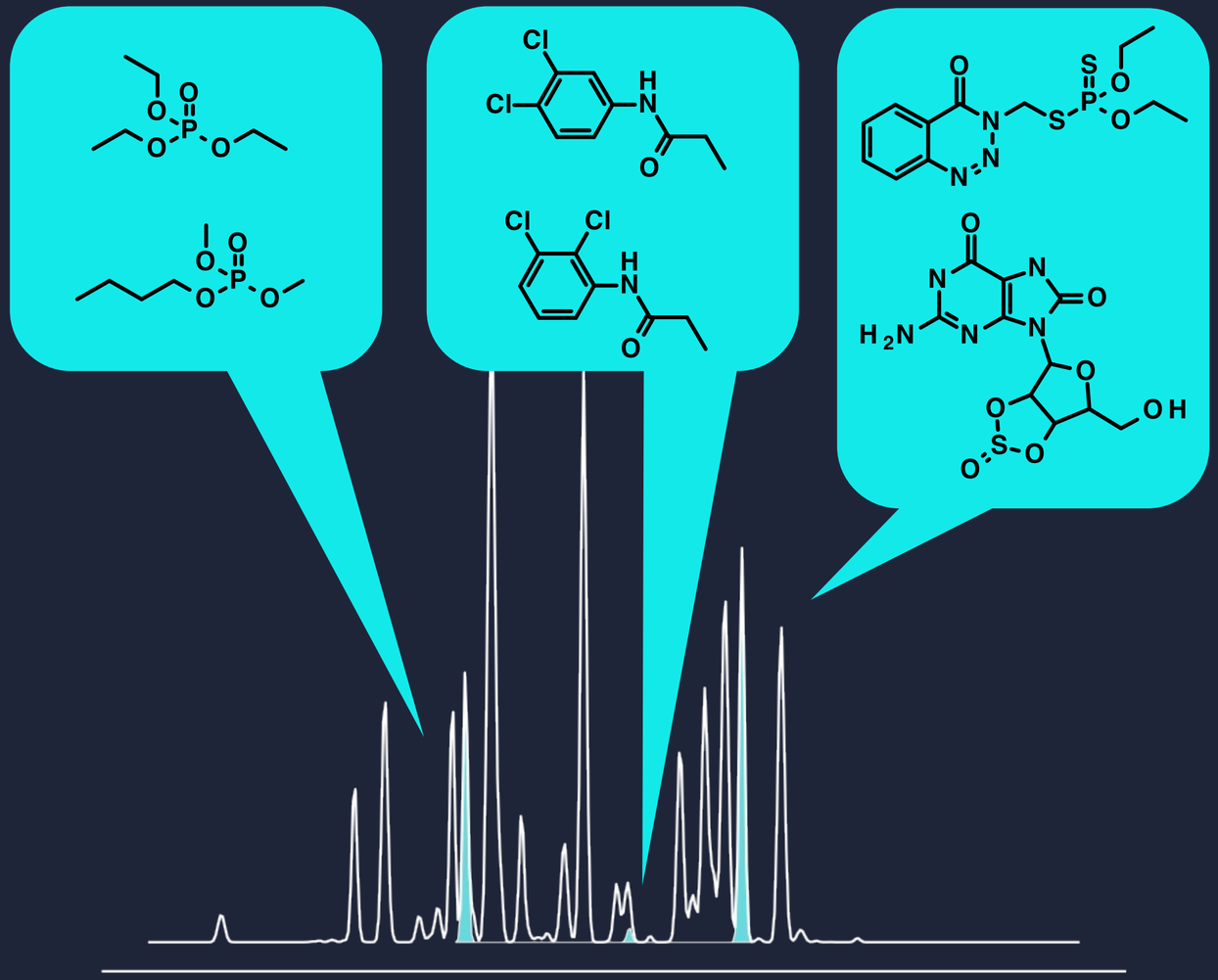
semi-supervised learning for RT

Sandberg et al. in preparation



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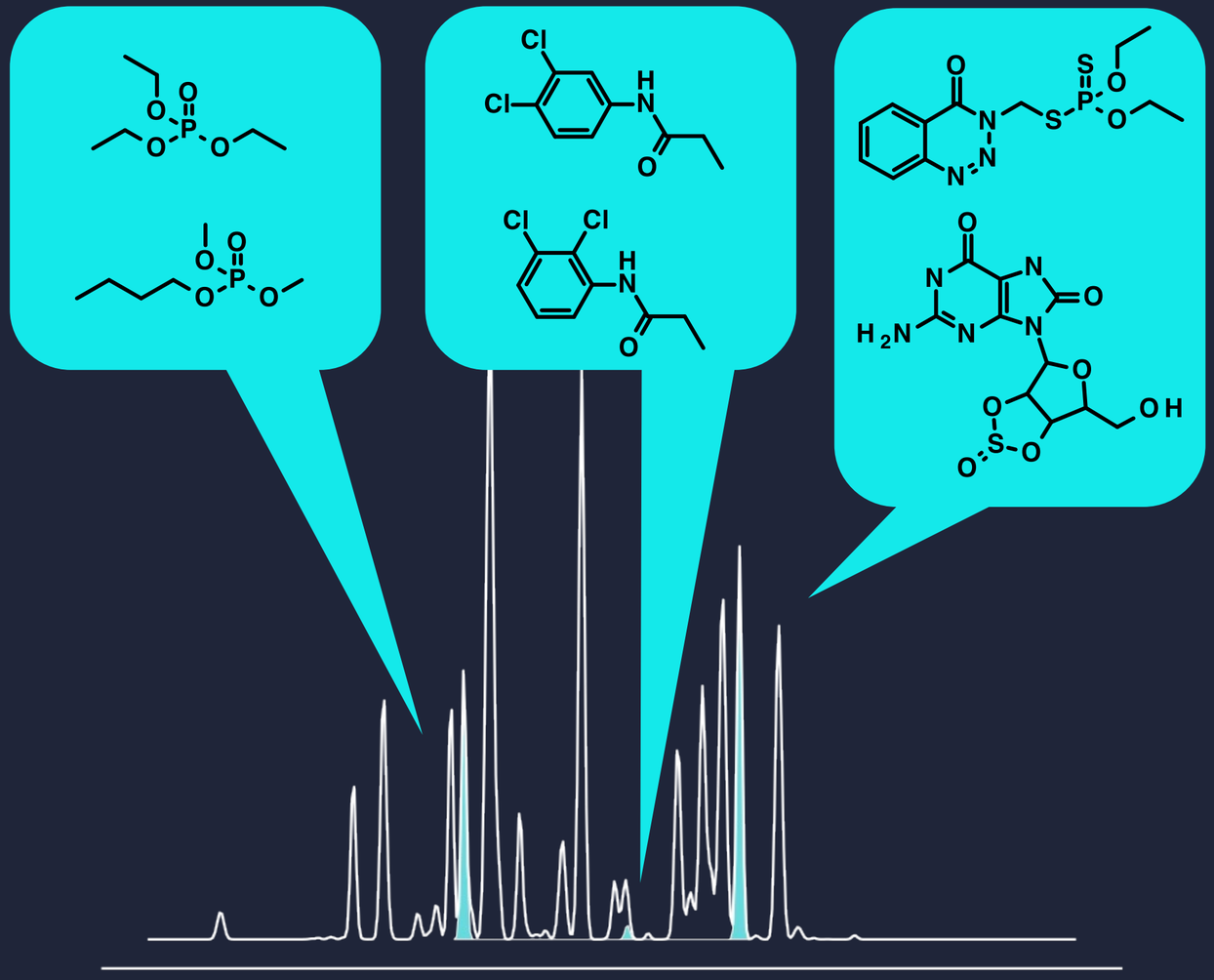

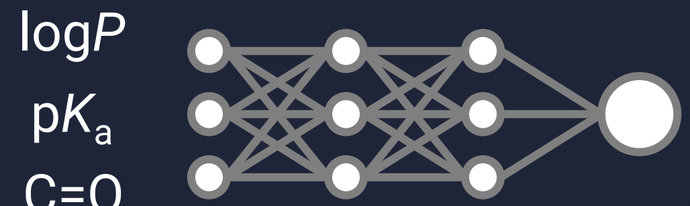

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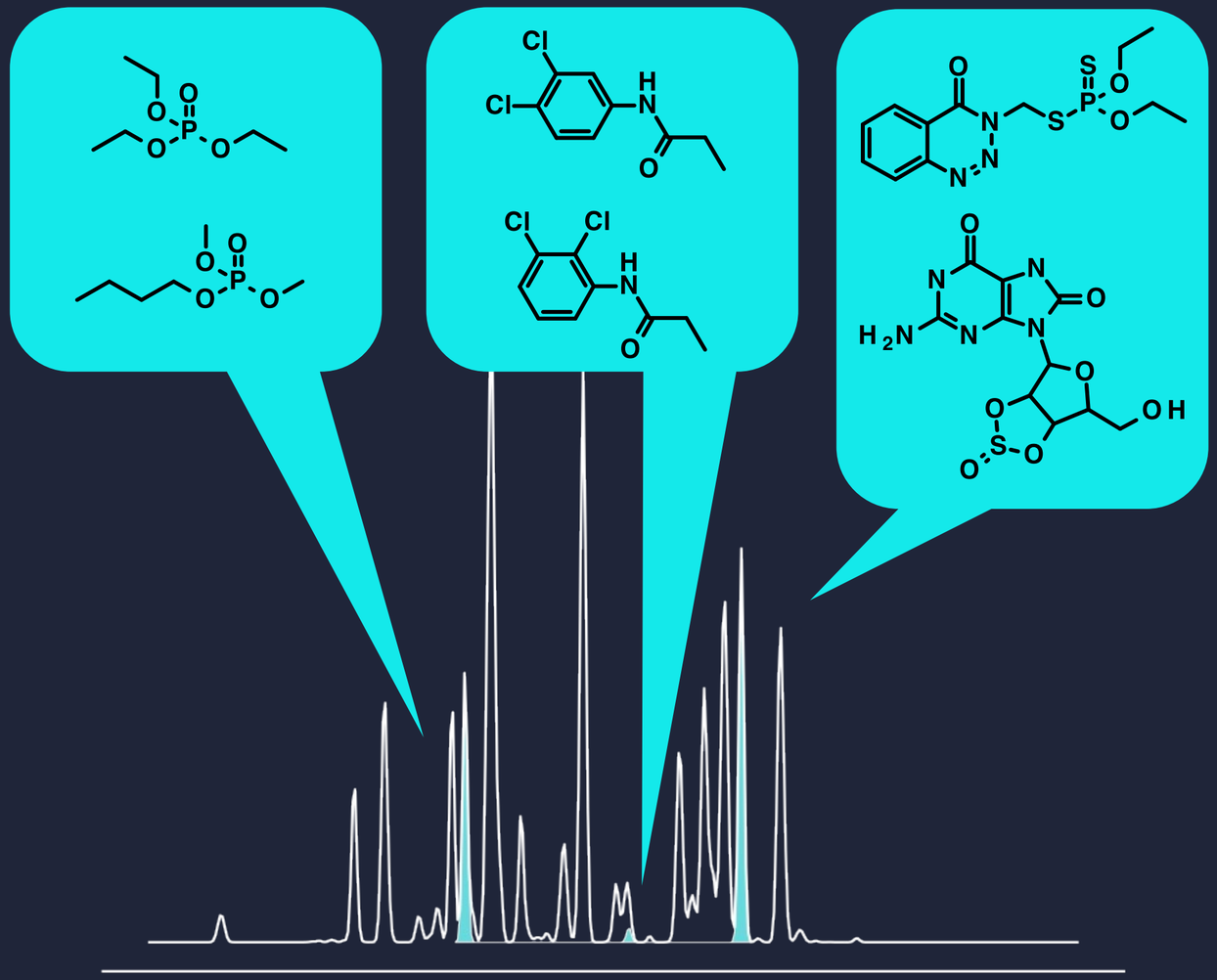
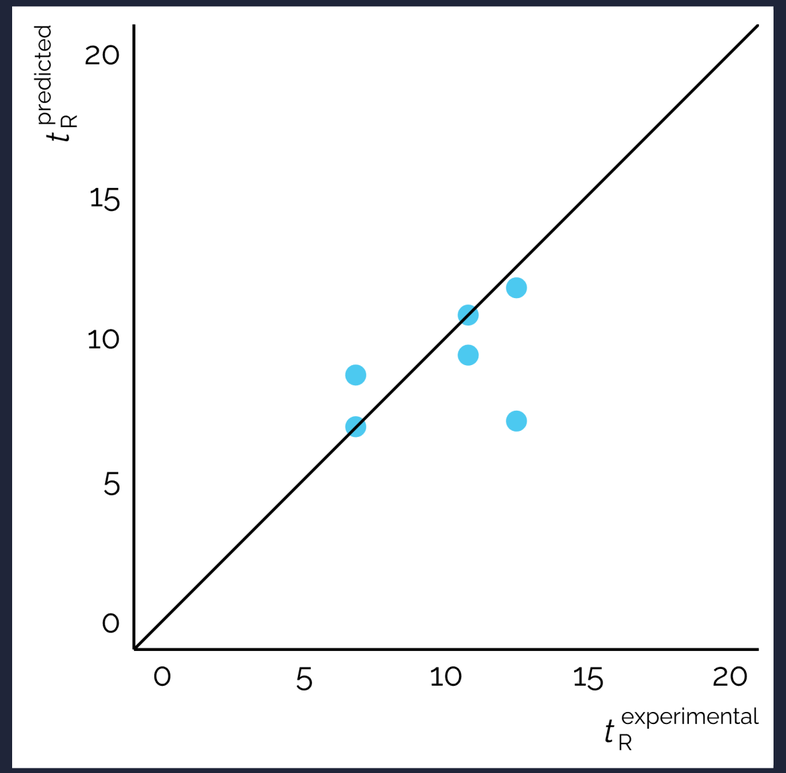
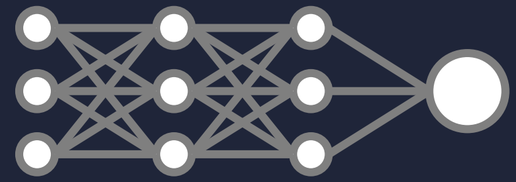




log*P*

pK_a

C=O



t_R

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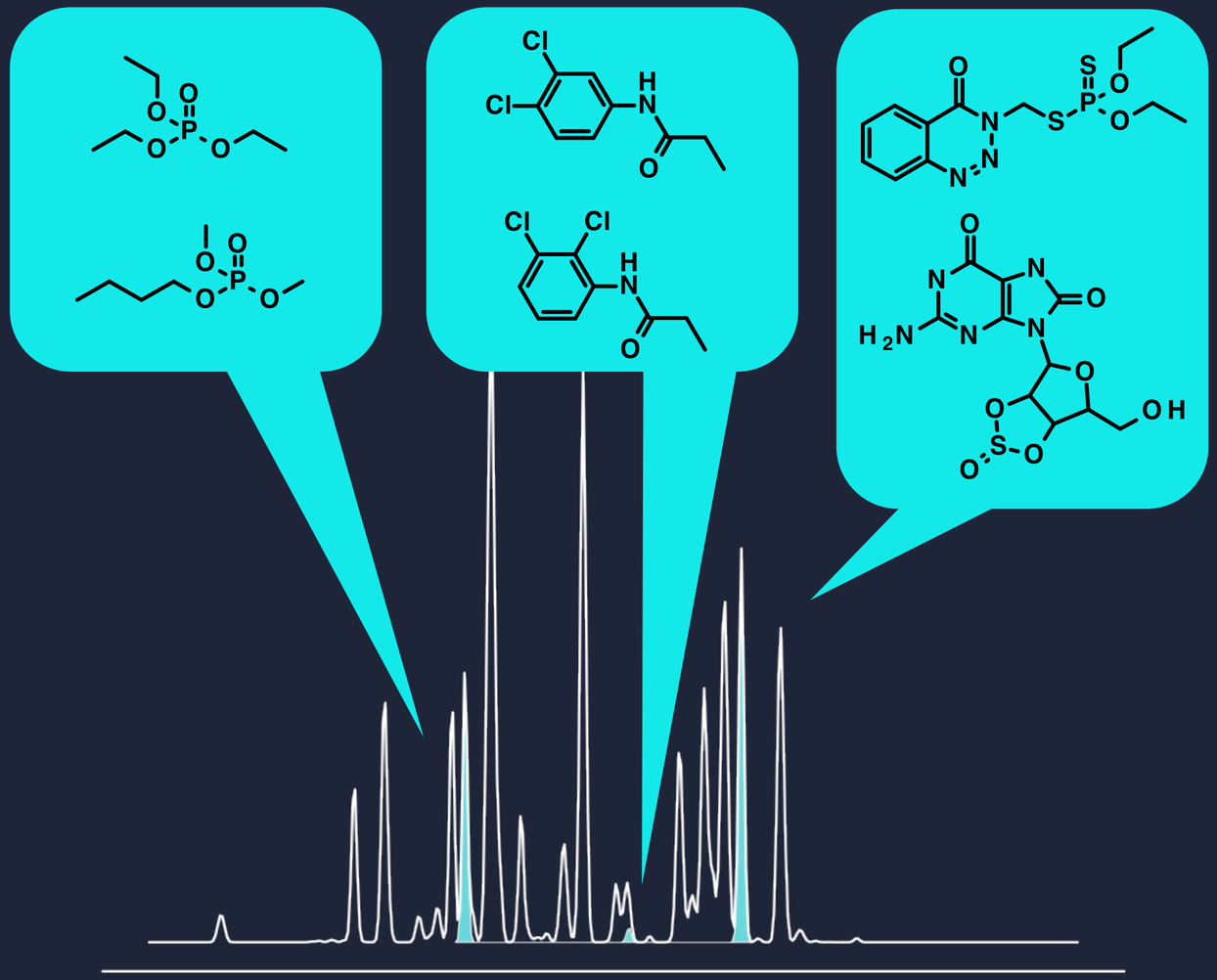

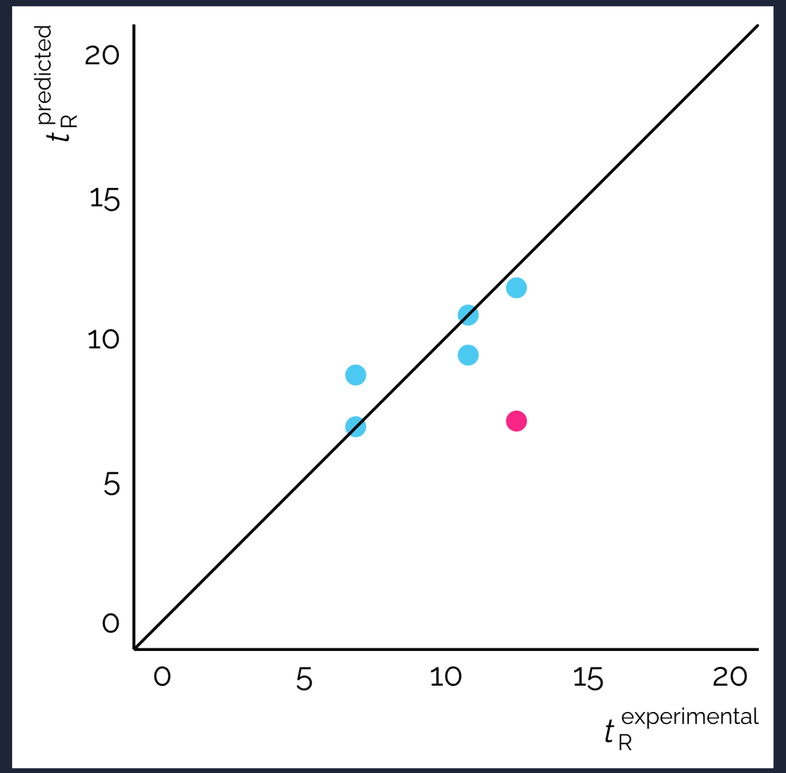

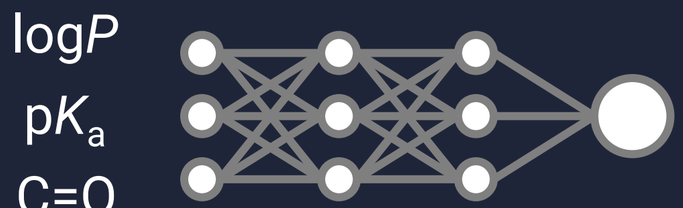

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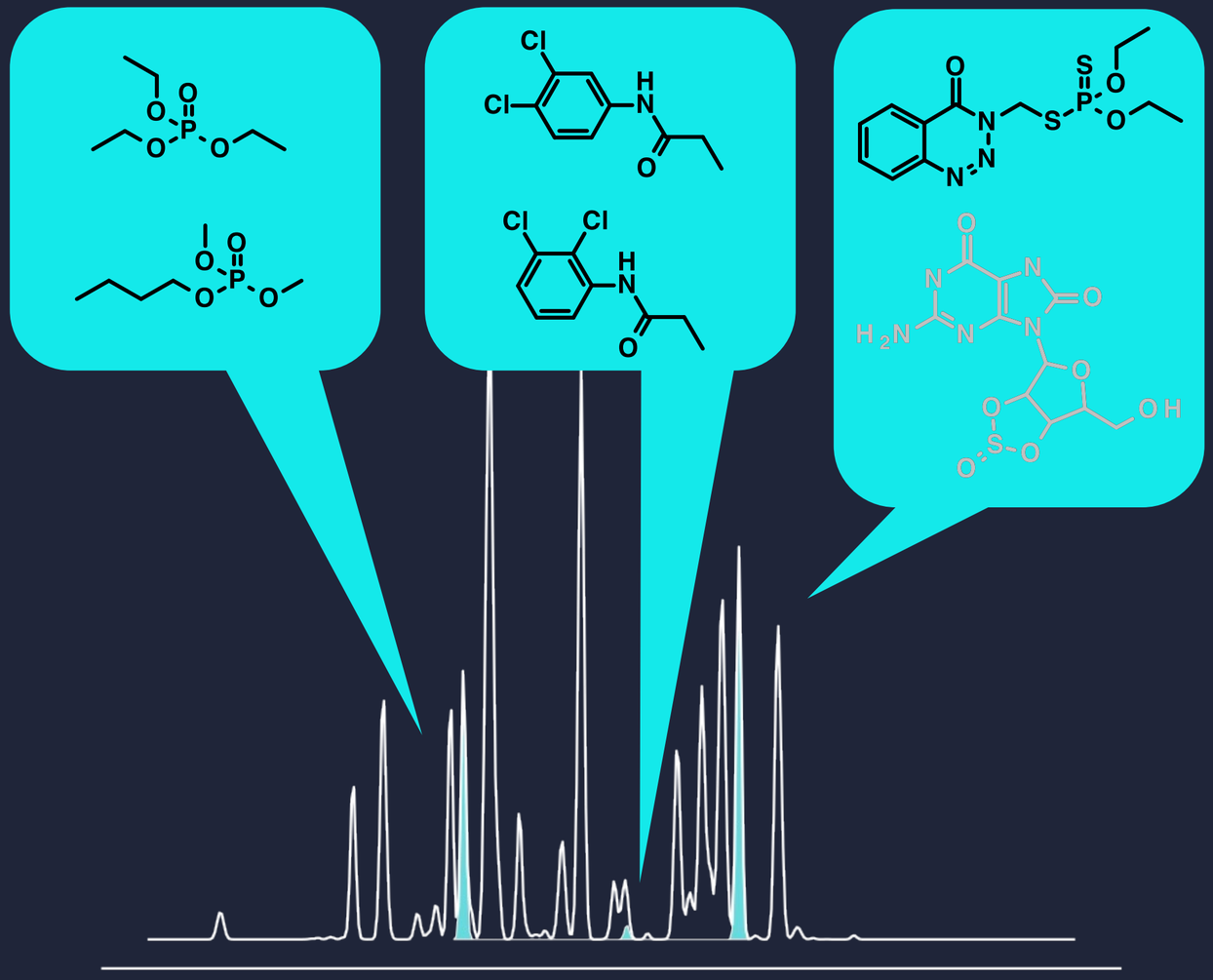

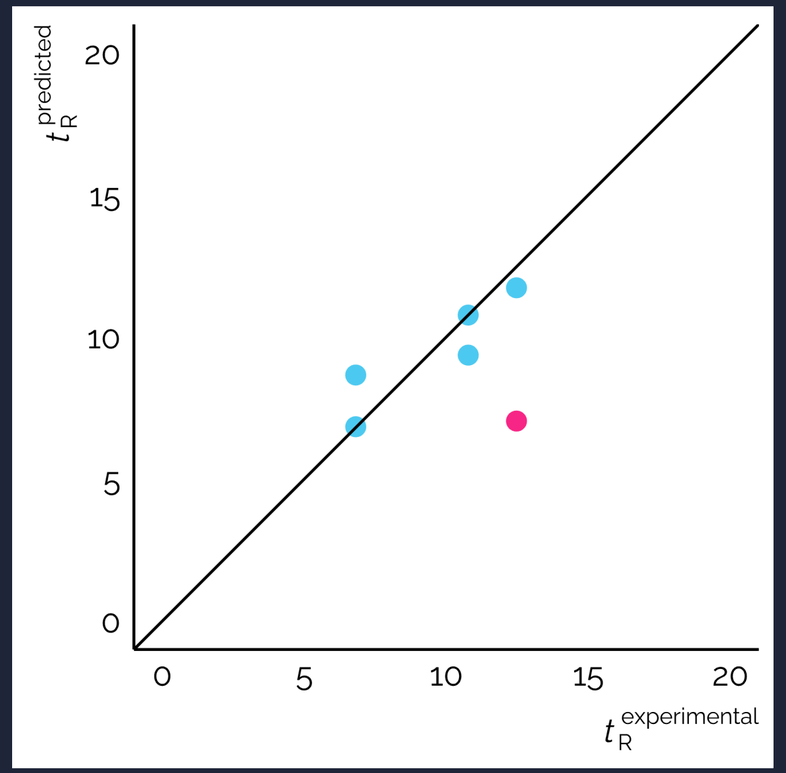

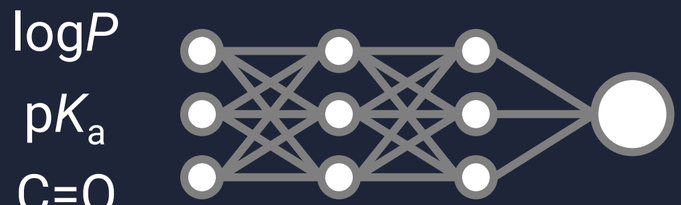

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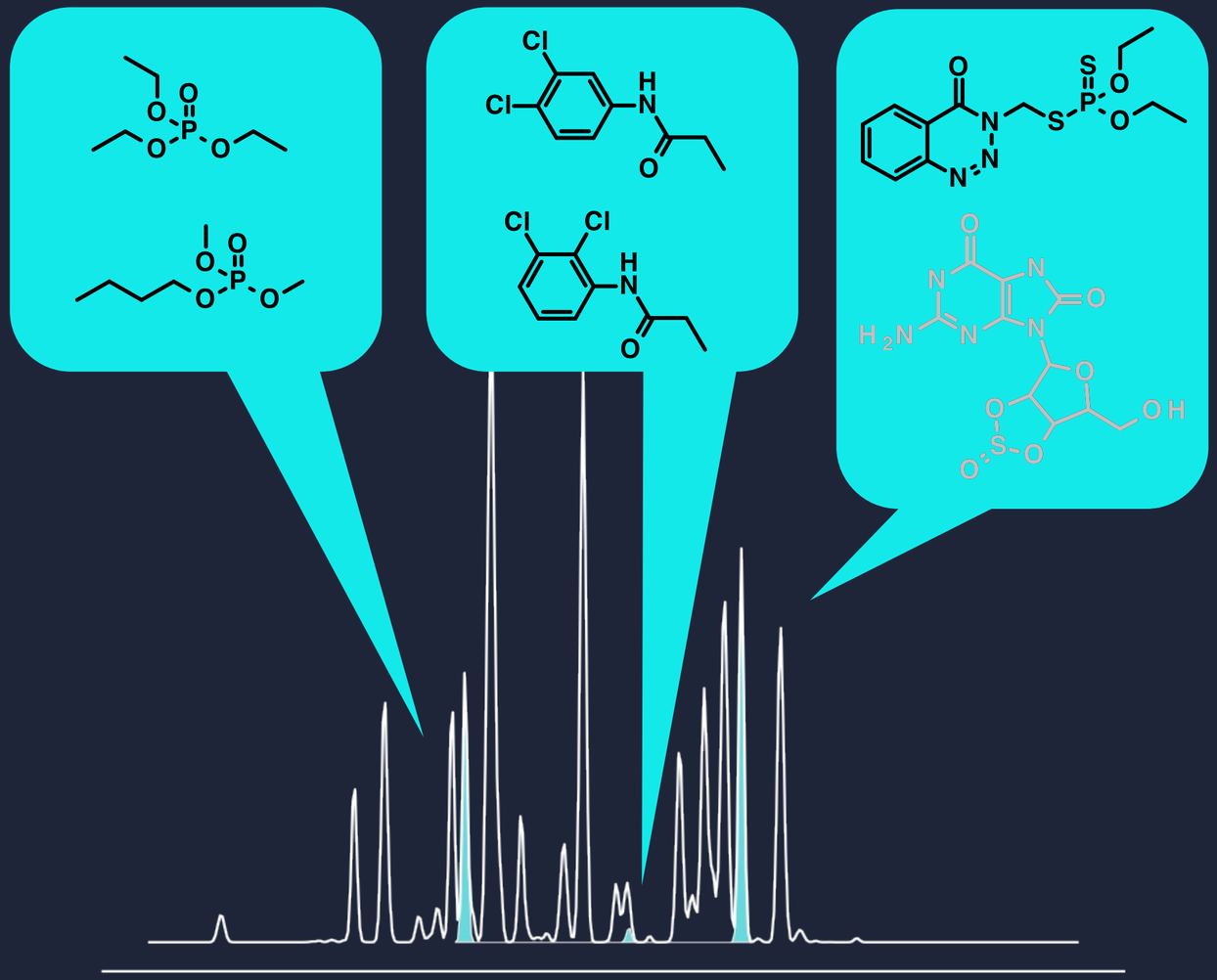
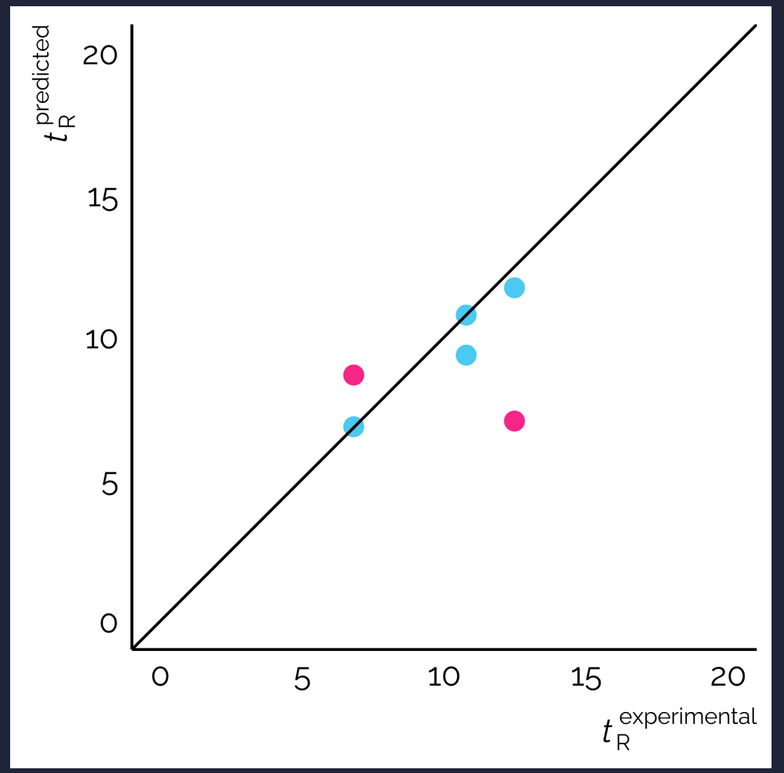
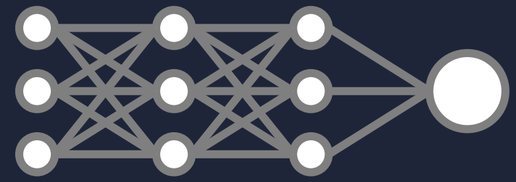




log*P*

pK_a

C=O



t_R

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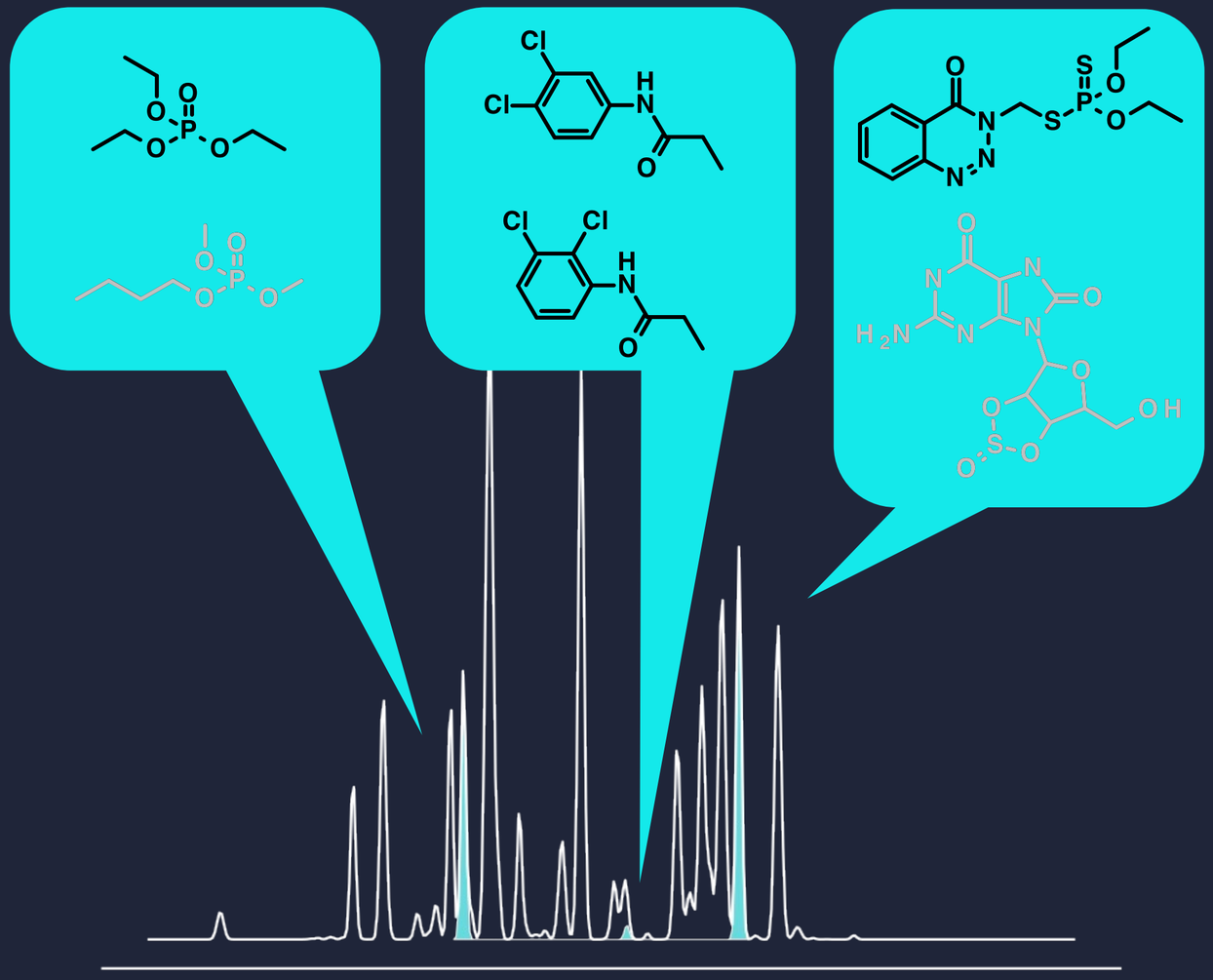

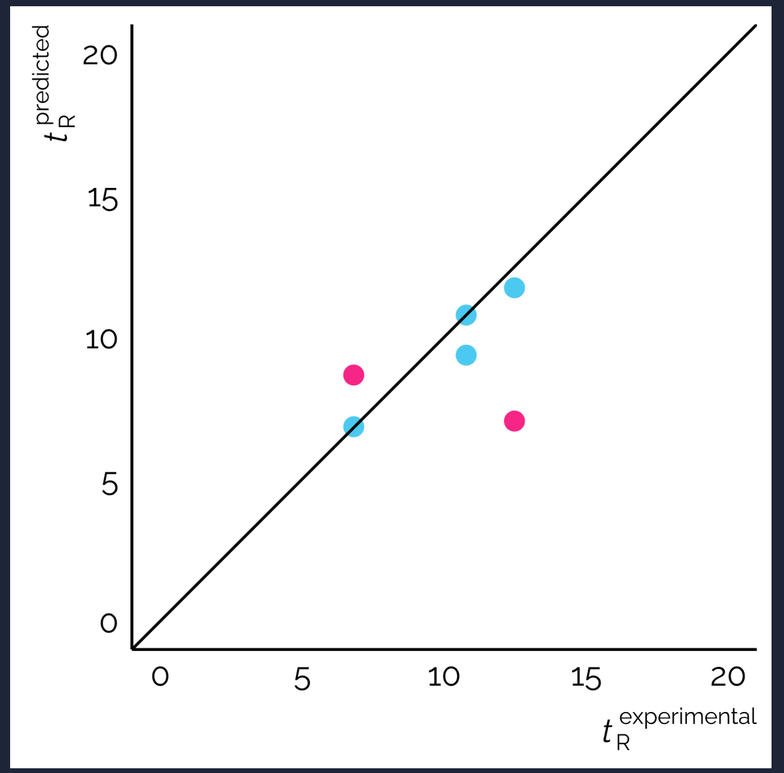

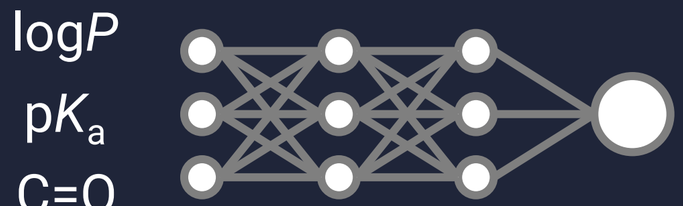

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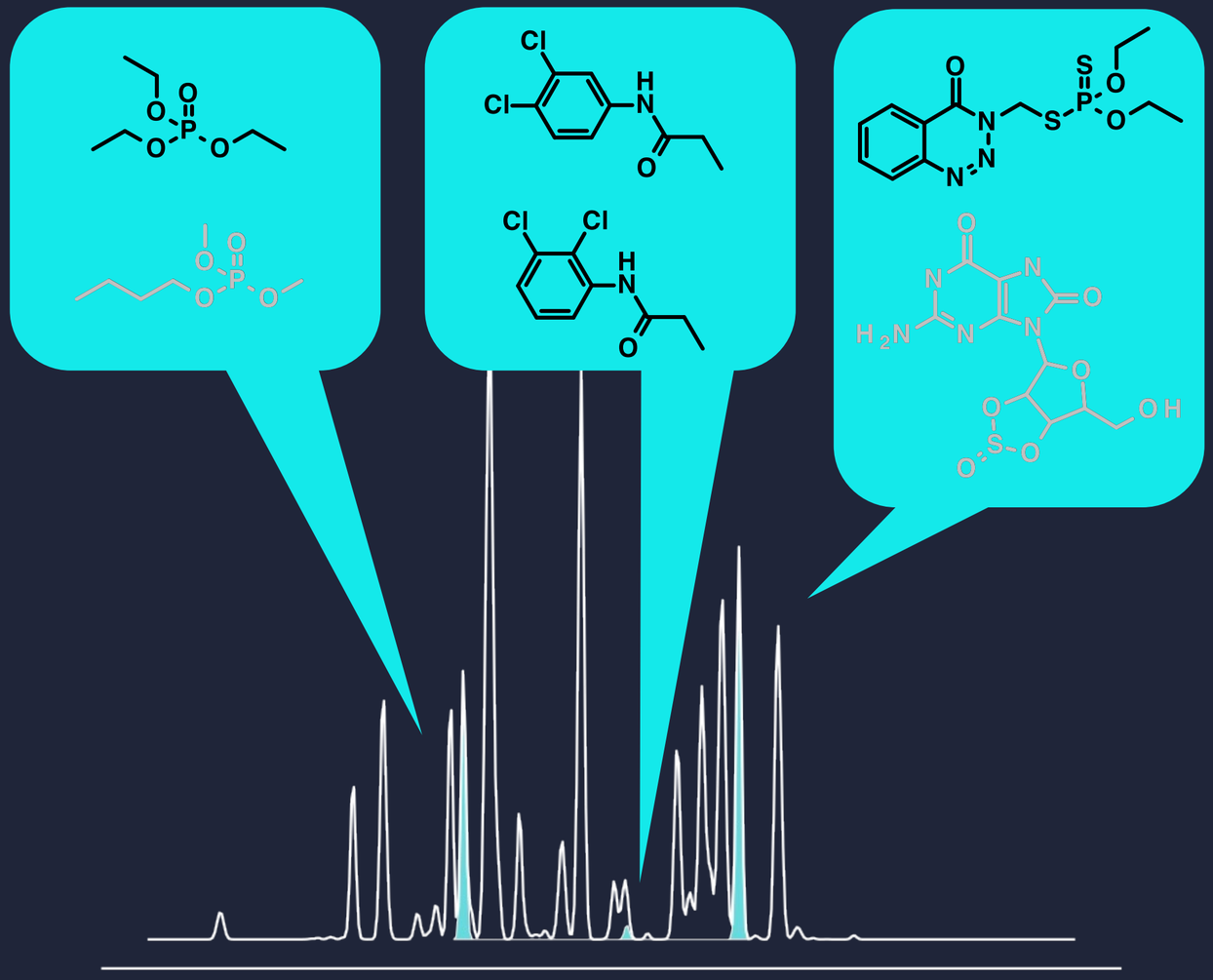
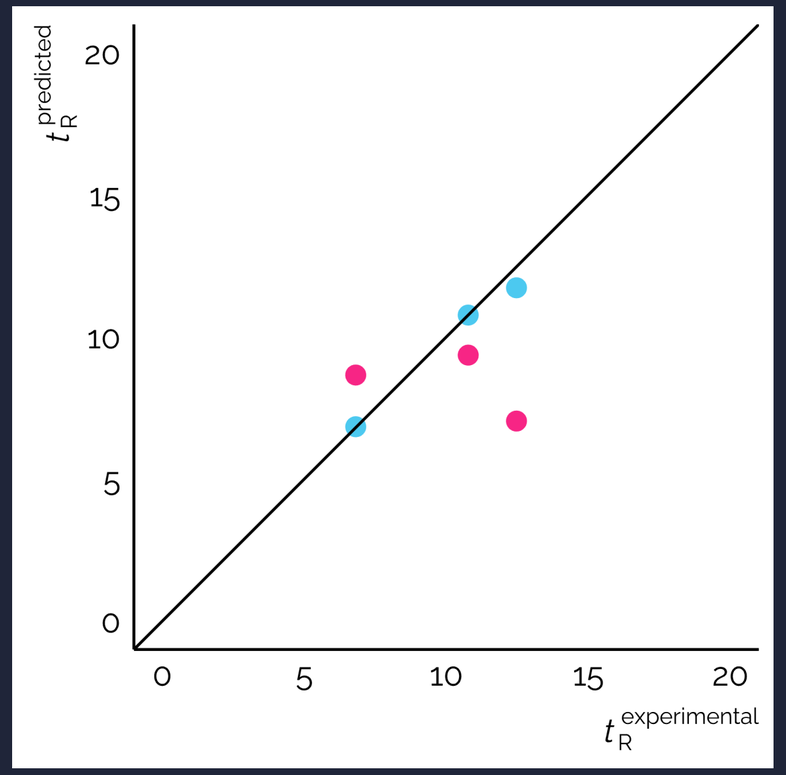
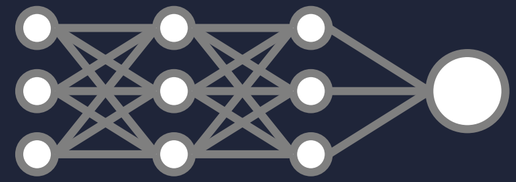




log*P*

pK_a

C=O



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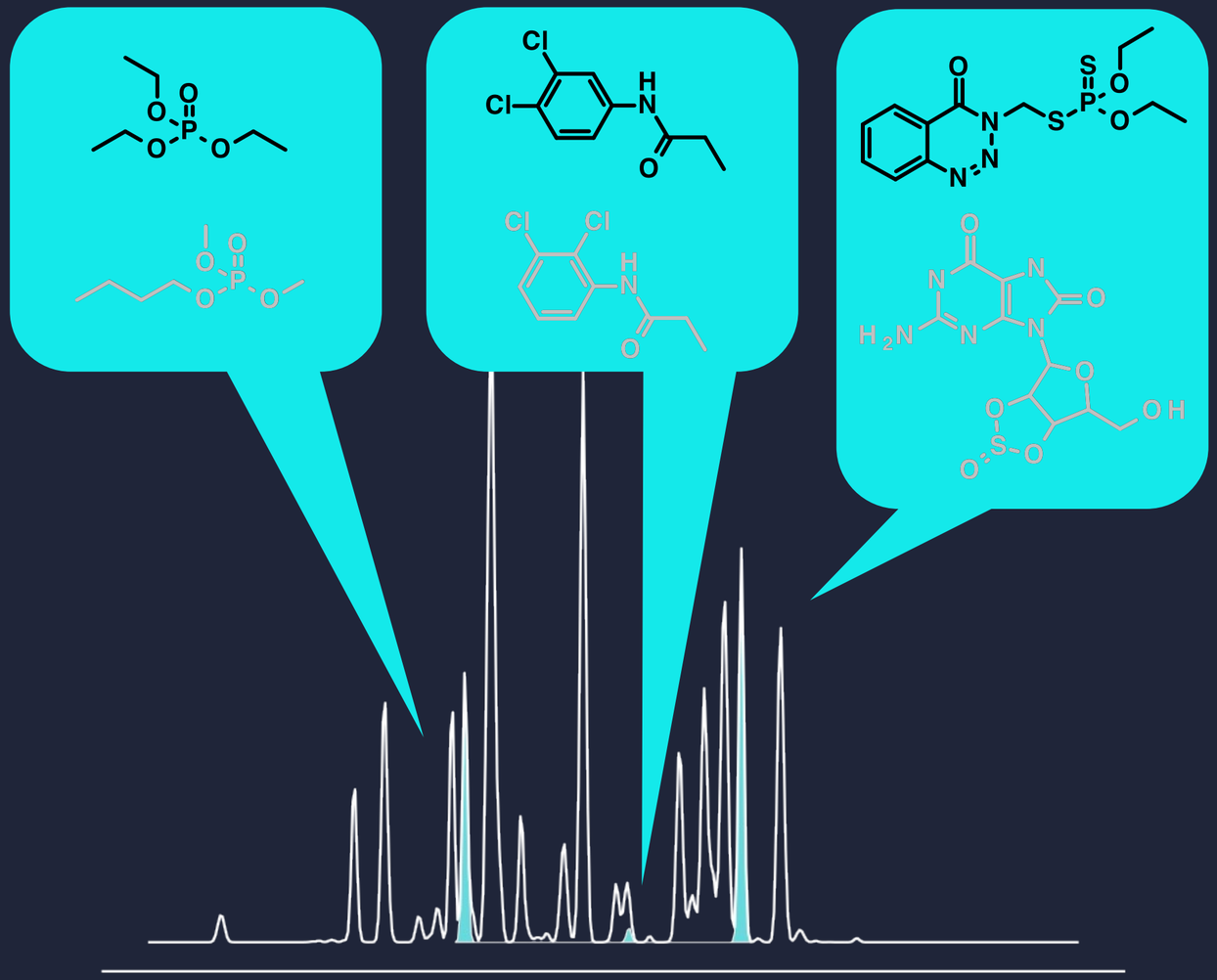

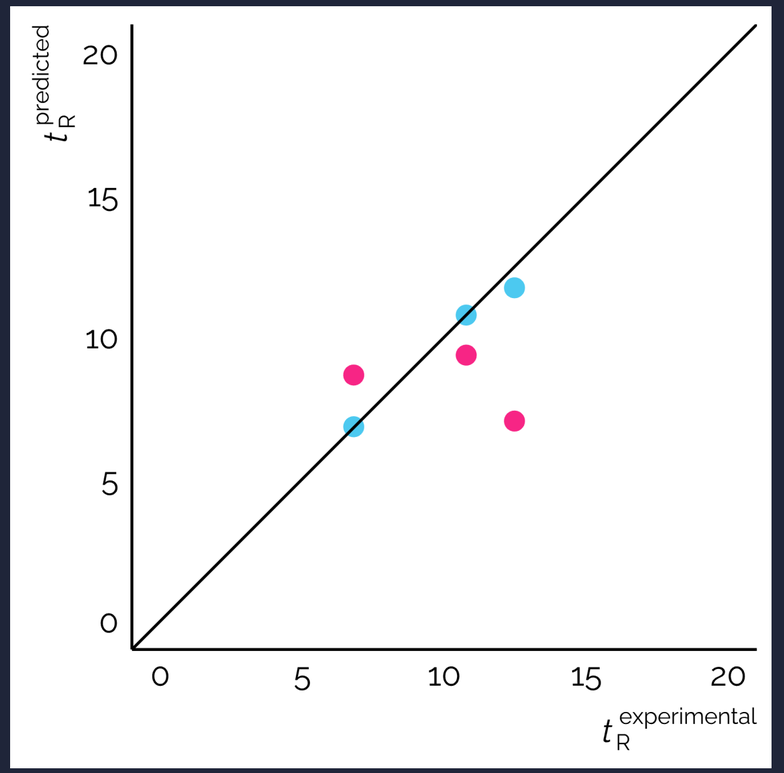

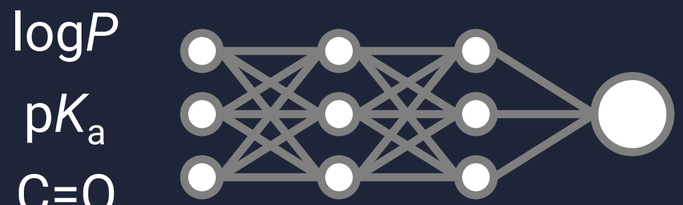

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candidate structure

evaluation

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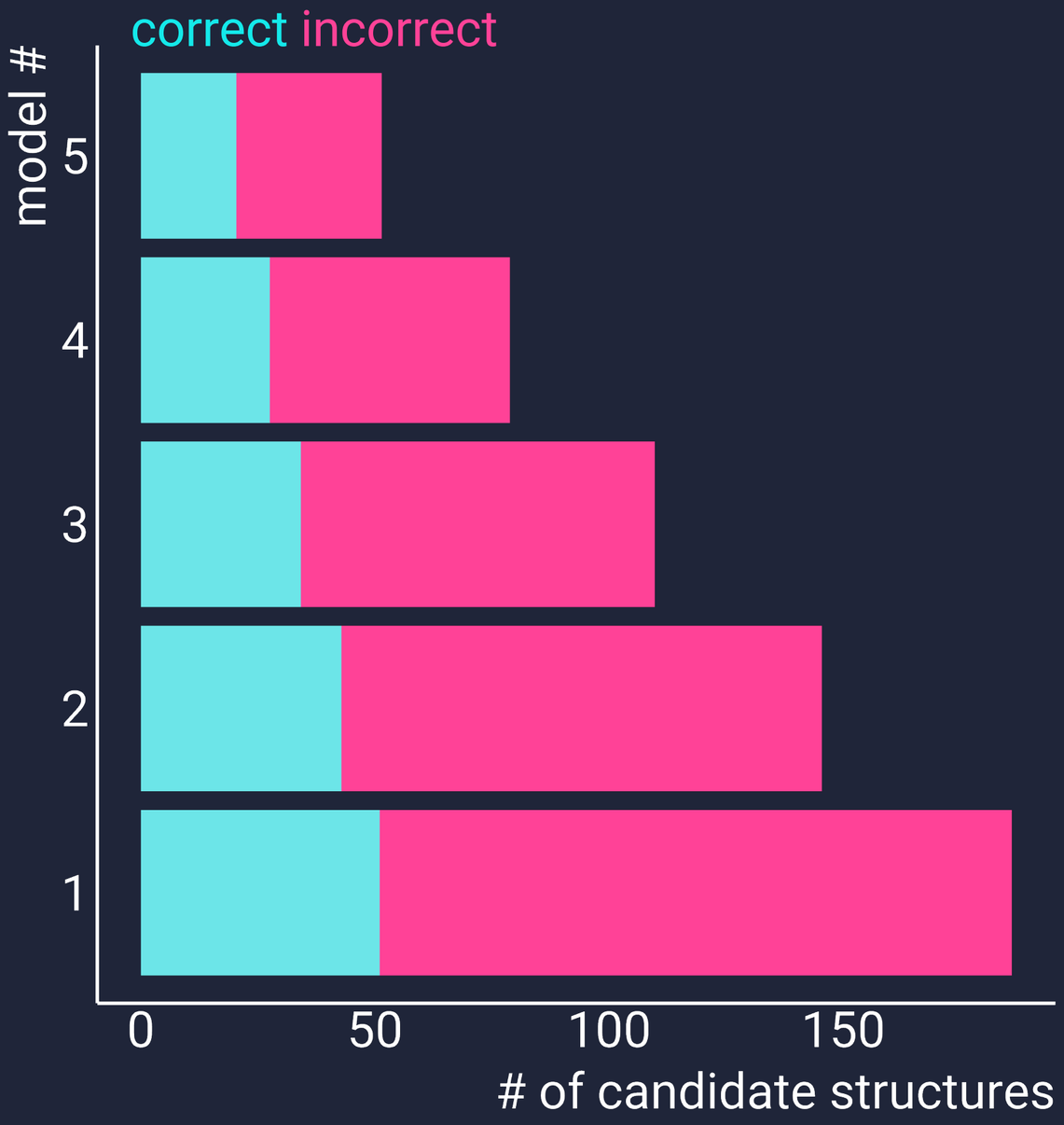

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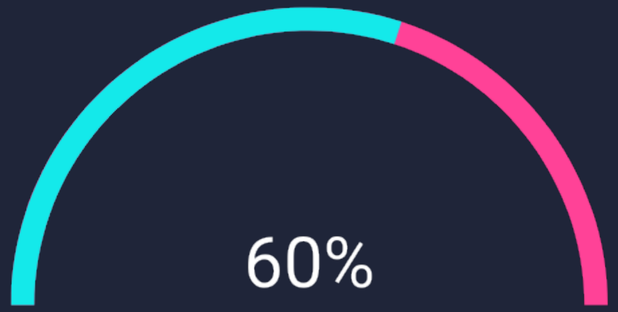
machine learning

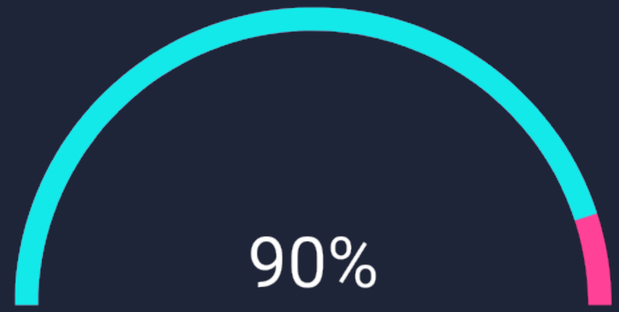
for prioritization and identification in nontarget screening

TOXICITY

QUANTIFICATION



RETENTION TIME



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Wallenberg Initiative Materials Science for Sustainability

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