patRoon 2.0: Non-target screening workflows for automated transformation product screening and other major improvements

Rick Helmus[a], Bas van de Velde[a,b,c], Andrea M. Brunner[b,d], Maarten R. van Bommel[e,f,g], Thomas L. ter Laak[a,b], Pim de Voogt[a], Annemarie P. van Wezel[a], Emma L. Schymanski[h]

[a] IBED, University of Amsterdam, the Netherlands
[b] KWR Watercycle Research Institute, the Netherlands
[c] AIMMS, Vrije Universiteit Amsterdam, the Netherlands
[d] EMSA, TNO, the Netherlands
[e] HIMS, University of Amsterdam, the Netherlands
[f] CASA, the Netherlands
[g] AHM, University of Amsterdam, the Netherlands
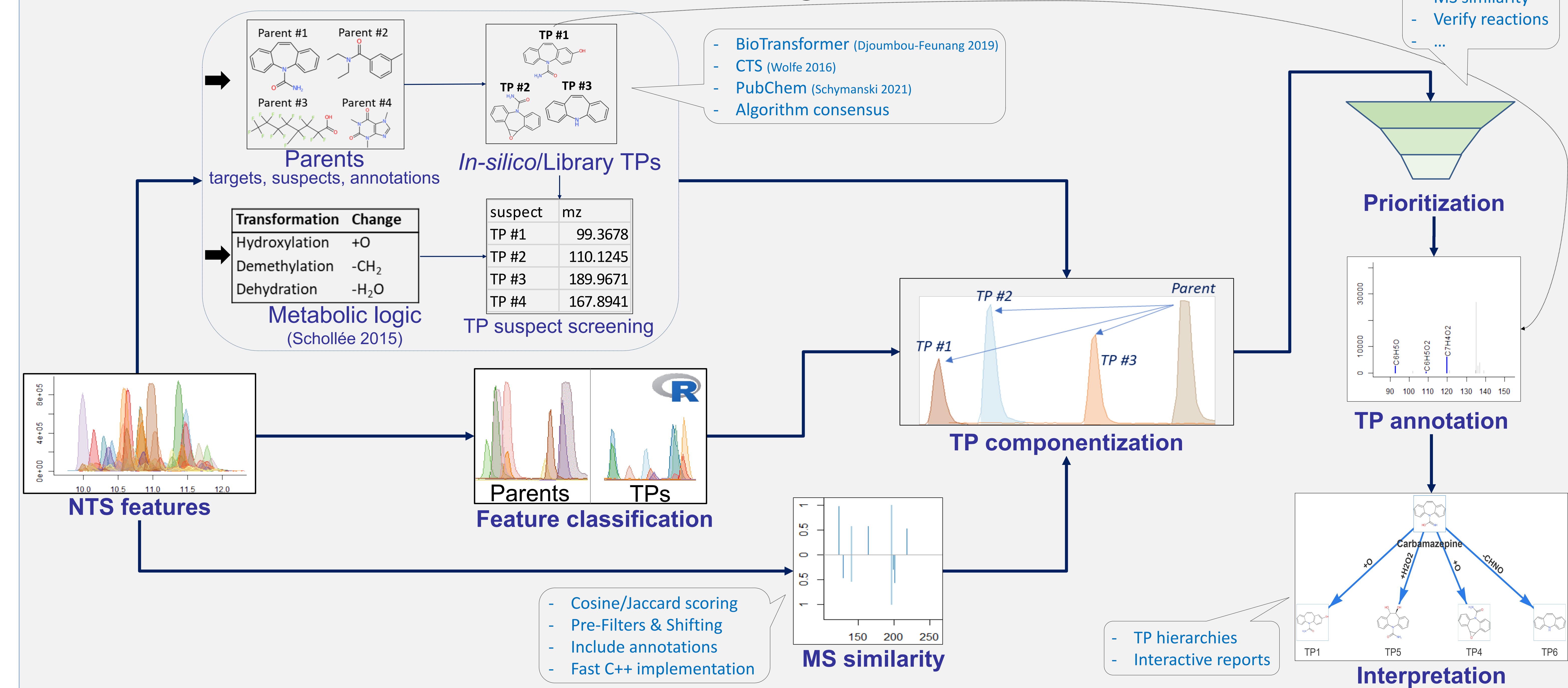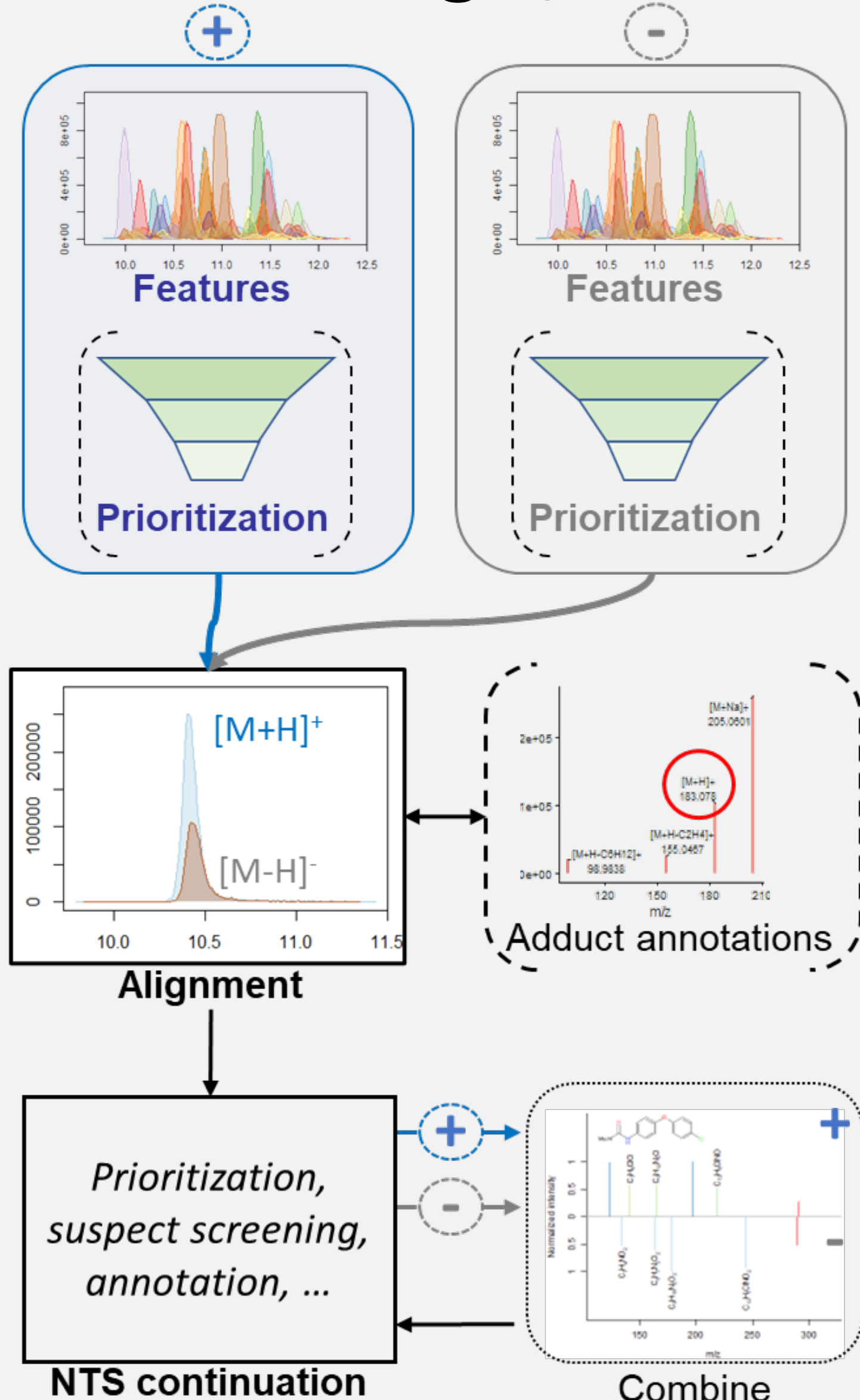[h] LCSB, University of Luxembourg, Belvaux, Luxembourg

Introduction

Non-target screening (NTS) with high resolution mass spectrometry is increasingly used to systematically identify chemicals of emerging concern (CECs) in the environment. Microbial degradation, UV irradiation and other processes can transform CECs into transformation products (TPs), which may pose similar or higher risk. However, the complexity of environmental samples and the lack of suitable workflows and data sources often complicates comprehensive identification of TPs. patRoon 2.0 is an open-source R based platform for comprehensive and flexible NTS workflows, now upgraded with automated TP screening and other novel functionality.
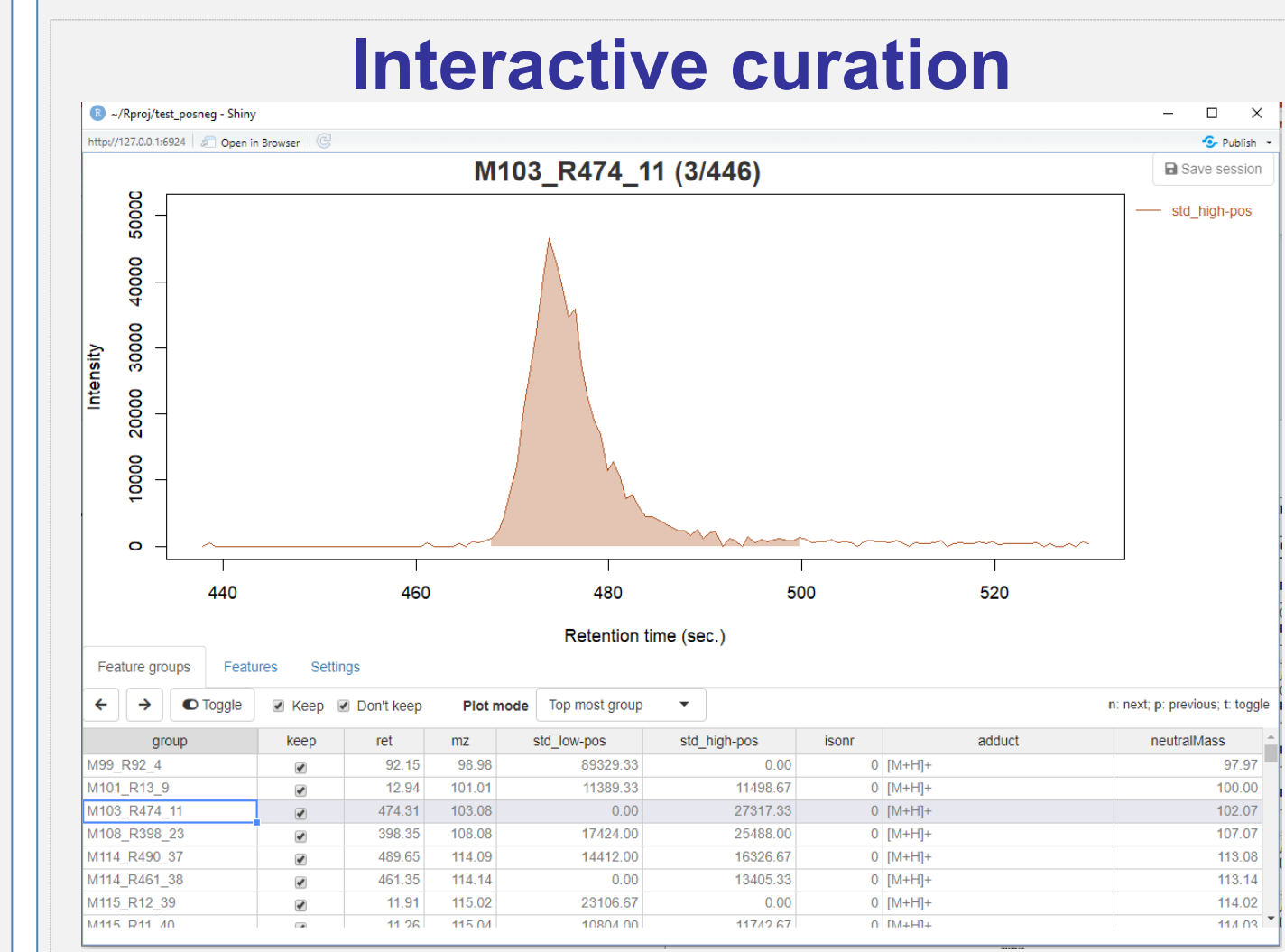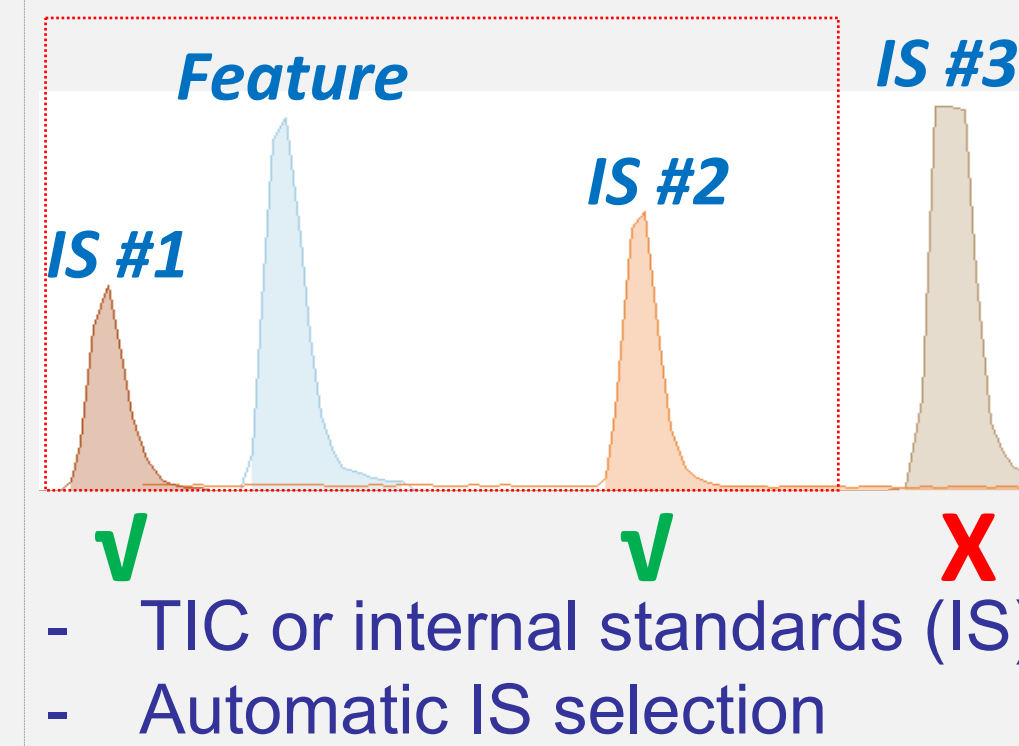
TP screening workflow

- Expected RT
- MS similarity
- Verify reactions
- ...

- BioTransformer (Djoumbou-Feunang 2019)
- CTS (Wolfe 2016)
- PubChem (Schymanski 2021)
- Algorithm consensus

Parents — targets, suspects, annotations

***In-silico*/Library TPs**

Transformation	Change
Hydroxylation	+O
Demethylation	-CH$_2$
Dehydration	-H$_2$O

Metabolic logic (Schollée 2015)

suspect	mz
TP #1	99.3678
TP #2	110.1245
TP #3	189.9671
TP #4	167.8941

TP suspect screening

Prioritization

TP annotation

NTS features

Feature classification — Parents / TPs

TP componentization

- Cosine/Jaccard scoring
- Pre-Filters & Shifting
- Include annotations
- Fast C++ implementation

MS similarity

- TP hierarchies
- Interactive reports

Interpretation

Combining +/- data

Features (+) / **Features** (−)

Prioritization

Alignment

Adduct annotations

Prioritization, suspect screening, annotation, …

NTS continuation

Combine

Other new functionality

Interactive curation

Feature normalization

IS #1, IS #2, IS #3, *Feature*
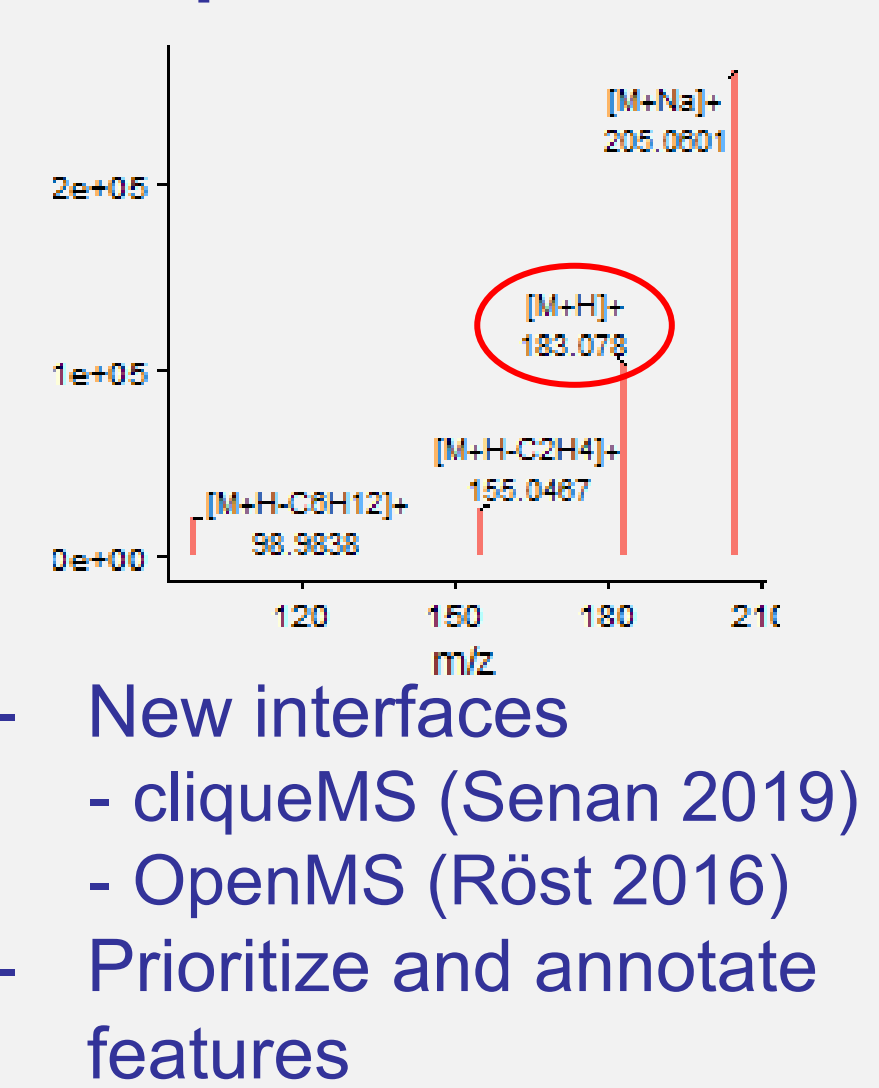
√ TIC or internal standards (IS)
- Automatic IS selection

MS libraries

MassBank — High Quality Mass Spectral Database

- MSP/JSON format
- Load, export, post-process
- Feature annotation

Improved componentization

- New interfaces
  - cliqueMS (Senan 2019)
  - OpenMS (Röst 2016)
- Prioritize and annotate features

Identification level estimation

Level	Criteria
1	Known RT & MS/MS
2a	Top ranked, high MS/MS library match & only candidate
3a	Fair MS/MS library match
3b	Known MS/MS fragments
3c	Good match in-silico MS/MS
4a	Top ranked formula, fair isotopic match & in-silico MS/MS match, no close candidates
4b	Top ranked formula, high isotopic match, no close candidates
5	All else

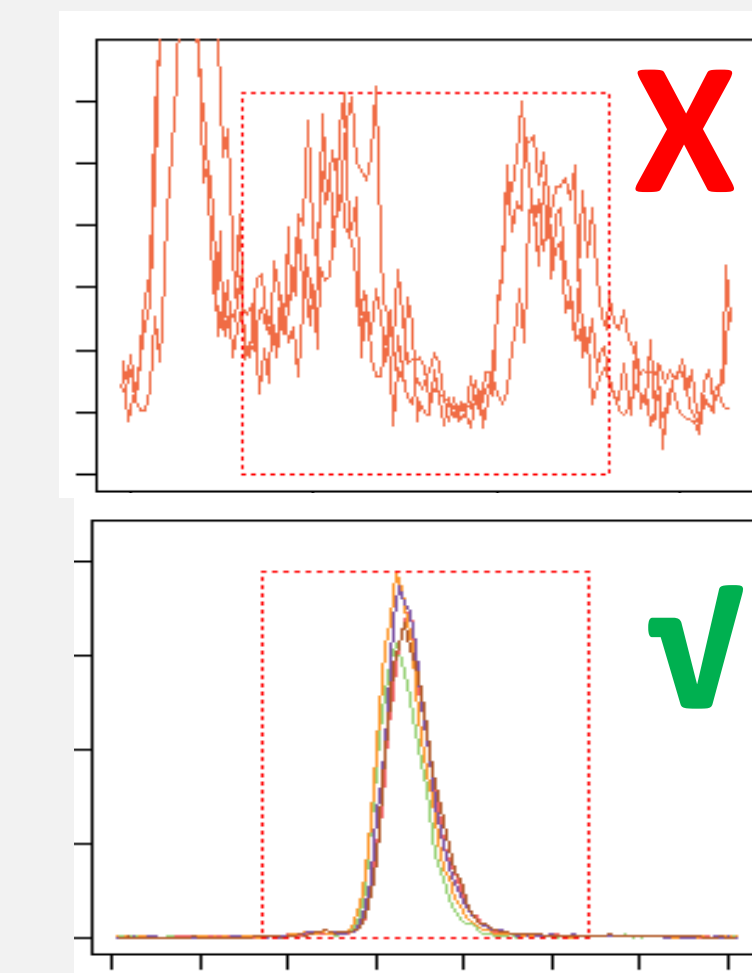Configurable rule based approach derived from Schymanski 2014

Extended feature detection
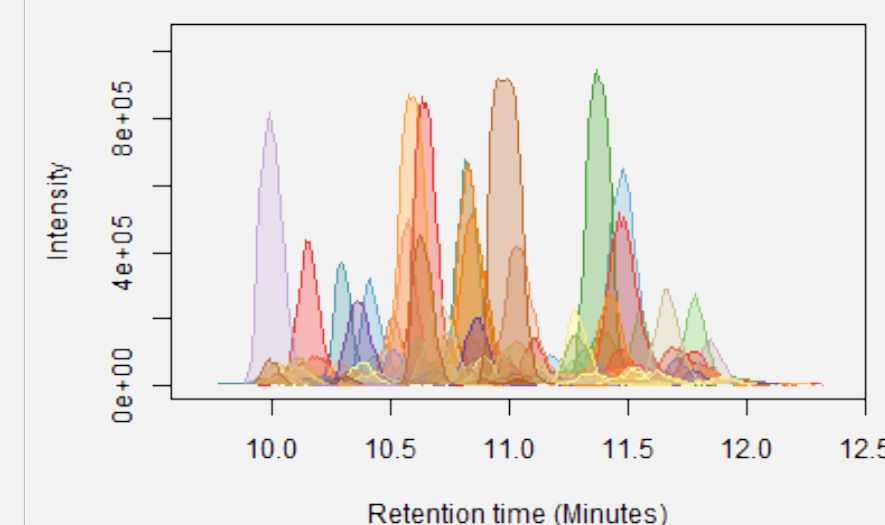
New interfaces to
- SIRIUS (Dührkop 2020)
- SAFD (Samanipour 2019)
- KPIC2 (Ji 2017)

Feature classification

Peak scoring and classification from MetaClean (Chetnik 2020)

patRoon

- Comprehensive NTA workflows
- Interfaces known algorithms
- Novel NTA functionality
- Instrument independent
- R/C++ based (open-source)
- Flexible & extendable
- Fully documented

Conclusions

- Automated workflows and multiple approaches to find and identify transformation products.
- Single workflow for +/- ionization data to simplify and improve data processing.
- Other major patRoon upgrades further extend and simplify NTS workflows.

Future applications

- Biotransformation product screening from CECs in wastewater effluent meant for re-use (*AquaConnect*)
- Elucidation of UV by-products formed during drinking water preparation processes (*TooCOLD*)
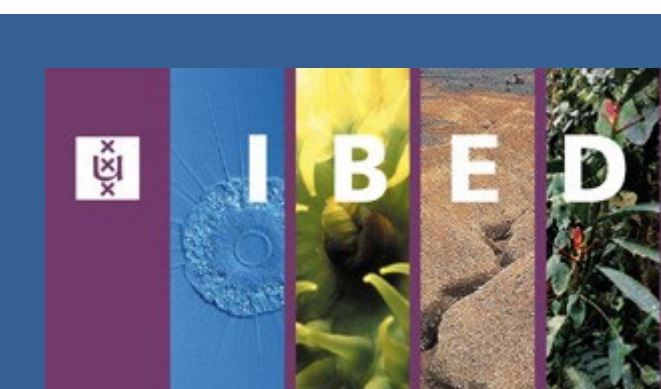
Contact
Rick Helmus
Institute for Biodiversity and Ecosystem Dynamics
Science Park 904, 1098 XH Amsterdam, the Netherlands

E: r.helmus@uva.nl
T: +31 20 525 6554