Non-targeted exploration of metabolic processes and xenobiotic metabolism in plants exposed to micropollutants using mass spectrometry imaging (MSI)

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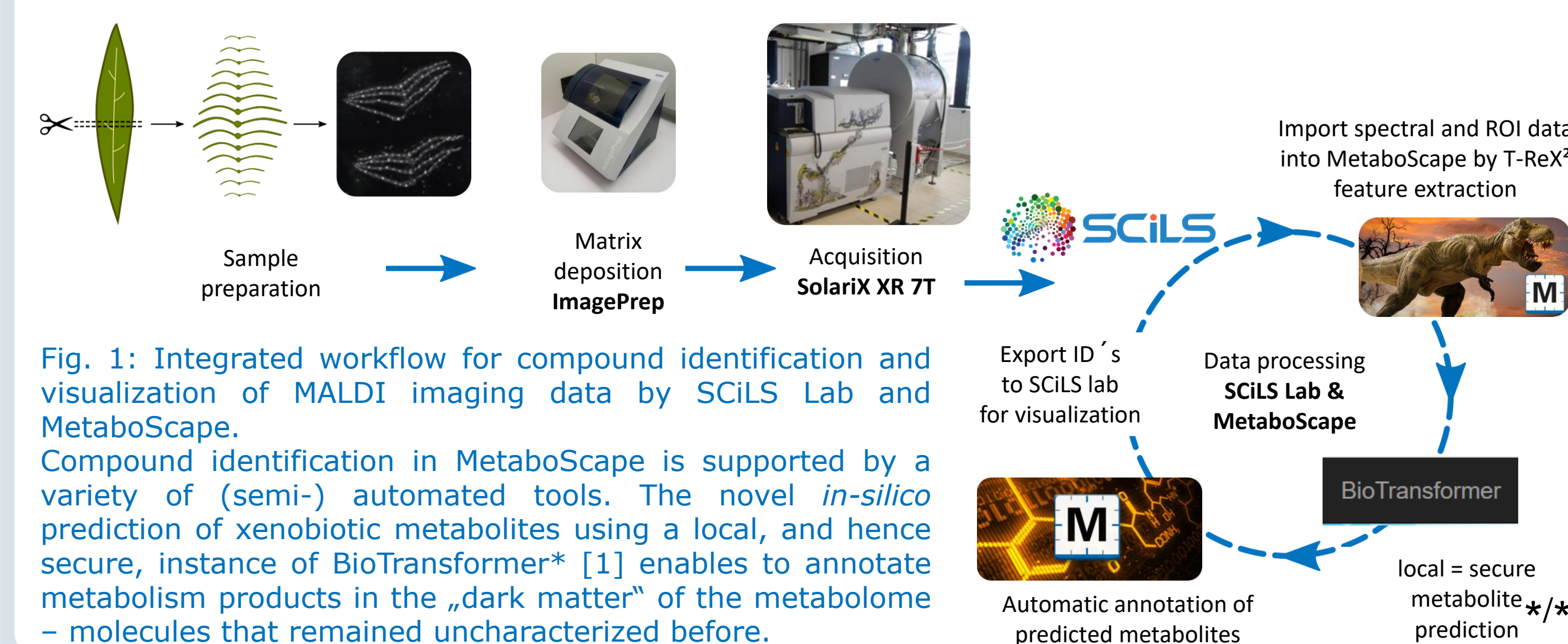[2]Bruker Daltonics, Bremen, Germany;
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Introduction

The targeted screening of pollutants in biological samples using LC-MS has been extensively developed. This resulted in quick and easy identification and quantification of pesticides, drugs, toxics or narcotics. The next step is to follow the metabolism of such compounds in living organisms: discover the spatial distributions of compounds and their derivatives (metabolites) in tissues and identify the enzymatic processes which might be involved in the transformation of these compounds. Here we present a workflow based on mass spectrometry imaging coupled to in-silico prediction of metabolites (biological derivatives) and automatic annotation. This enabled the investigation of metabolic processes occurring when living organisms are exposed to pollutants, or more generally to xenobiotics (compounds from exogenous origin).

SCiLS and MetaboScape:
Tailored molecular imaging workflow for ID and visualization

Fig. 1: Integrated workflow for compound identification and visualization of MALDI imaging data by SCiLS Lab and MetaboScape.
Compound identification in MetaboScape is supported by a variety of (semi-) automated tools. The novel in-silico prediction of xenobiotic metabolites using a local, and hence secure, instance of BioTransformer* [1] enables to annotate metabolism products in the „dark matter" of the metabolome – molecules that remained uncharacterized before.

References & Notes

[1] Djoumbou-Feunang et al.; Journal of Cheminformatics 2019, 11:2

[2] Villette et al.; Metabolomics 2019, 15:122

[3] Wickler et al.; Nucleic Acid Research 2016, D502-D508

*BioTransformer is not a Bruker products and restrictions to utilize this software tool might apply or might change without notice from Bruker, please refer to the BioTransformer page for details - https://bitbucket.org/djoumbou/biotransformerjar/src/master/.

**Environmental microbial prediction is available at http://biotransformer.ca/. But: The use of the Environmental Microbial Prediction requires a dedicated license for commercial use which can be obtained from EnviPath: https://envipath.com/

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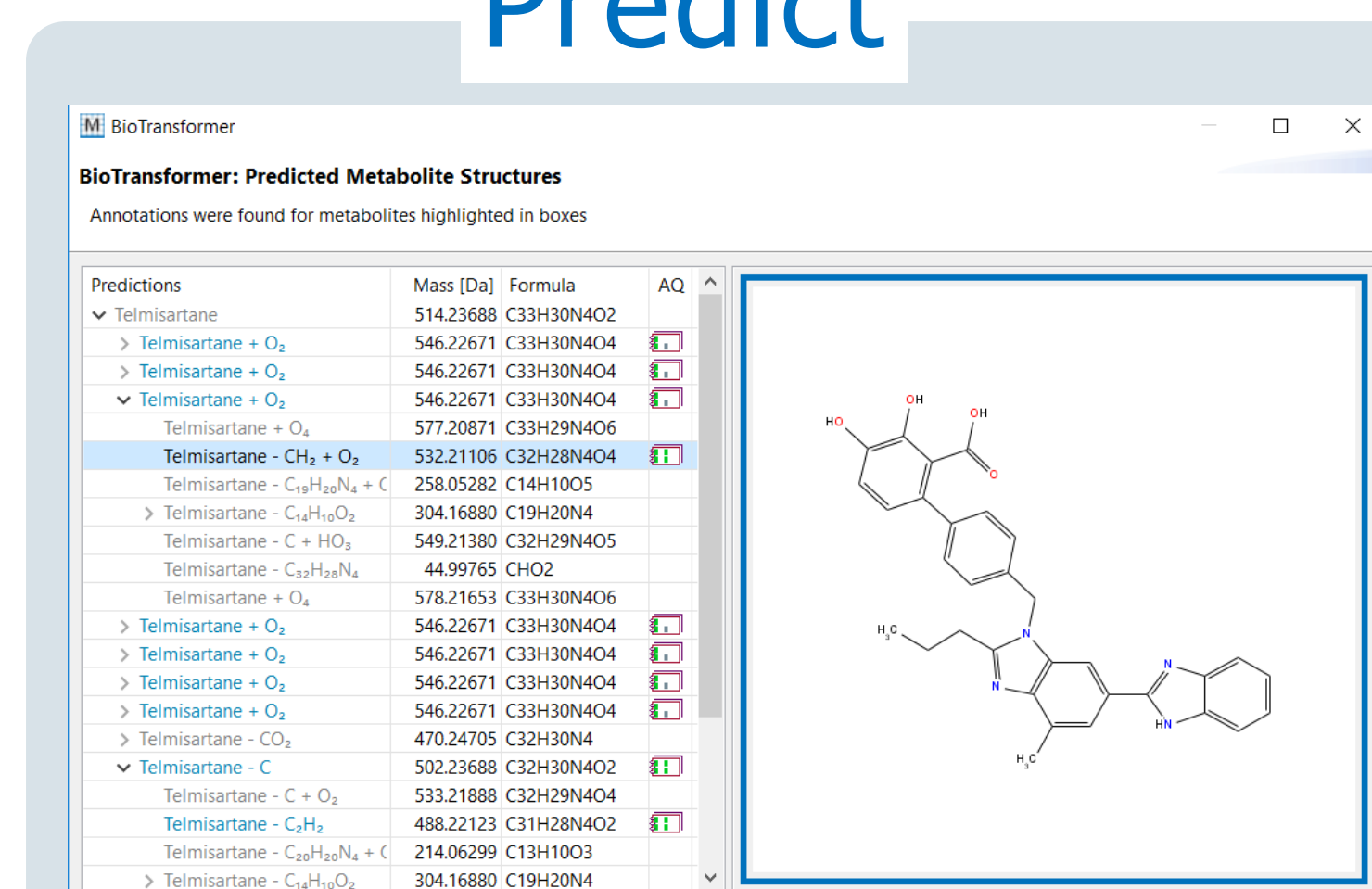
Predict

Fig. 2 Automatic prediction of Telmisartan metabolites using the "SuperBio" and "Environmental Microbial Transformation**" BioTransformer* modes to mimic metabolism of small molecules in humans and environmental microbial degradation, respectively. Predicted metabolites which were detected in the MSI data are highlighted in blue and the Annotation Quality scoring (AQ) is shown for quickly assessing confidence in ID.
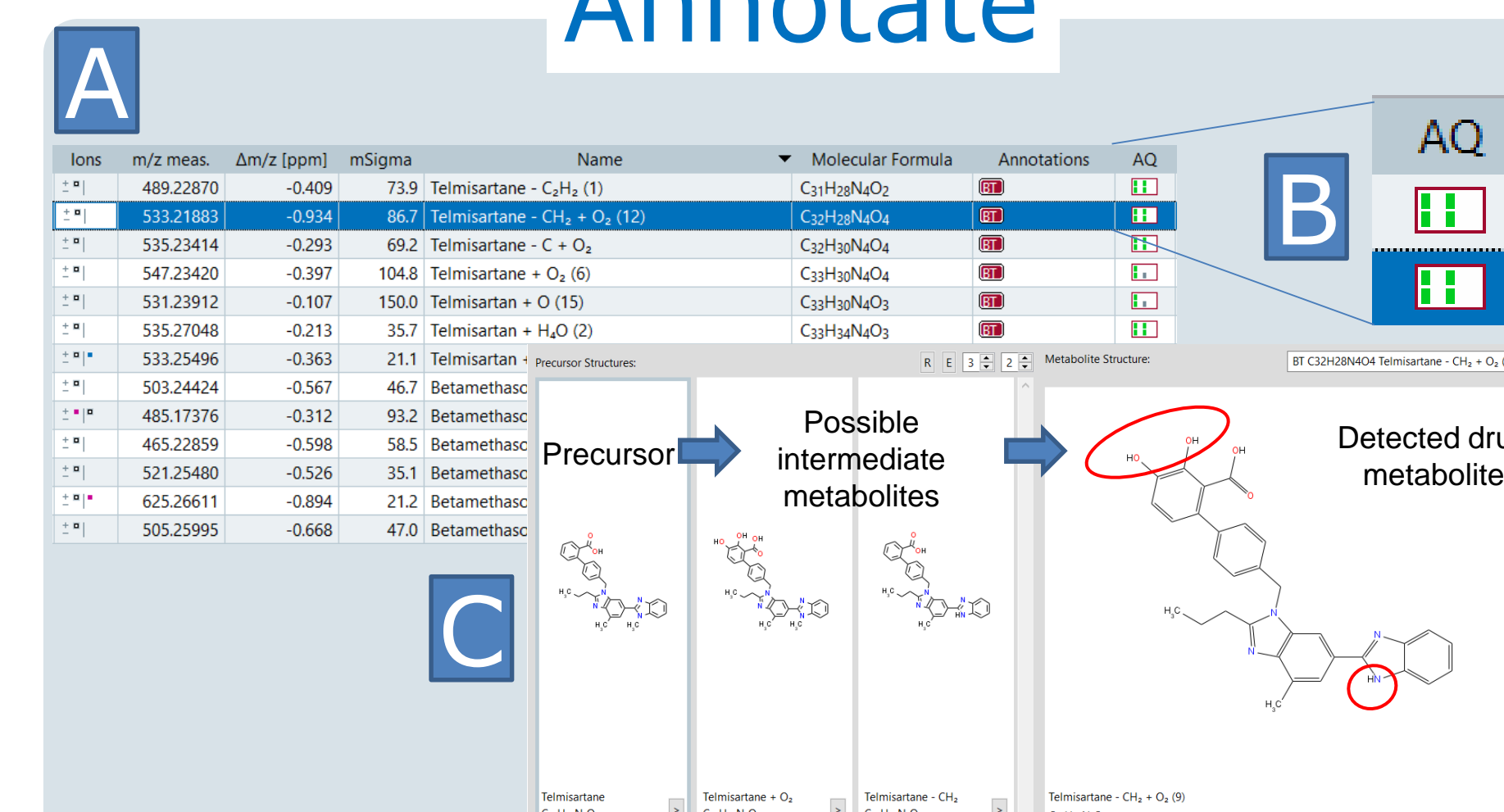
Annotate

Fig. 3 A) Annotation is performed for the predicted metabolites, taking into account quality scores based on m/z, isotope pattern. B) To assess the confidence in any annotation with a glimpse, matching criteria are reported in a concise but detailed summary, called the Annotation Quality AQ Scoring. C) Selecting an annotated feature highlights a matching metabolite structure and the corresponding metabolite precursors.
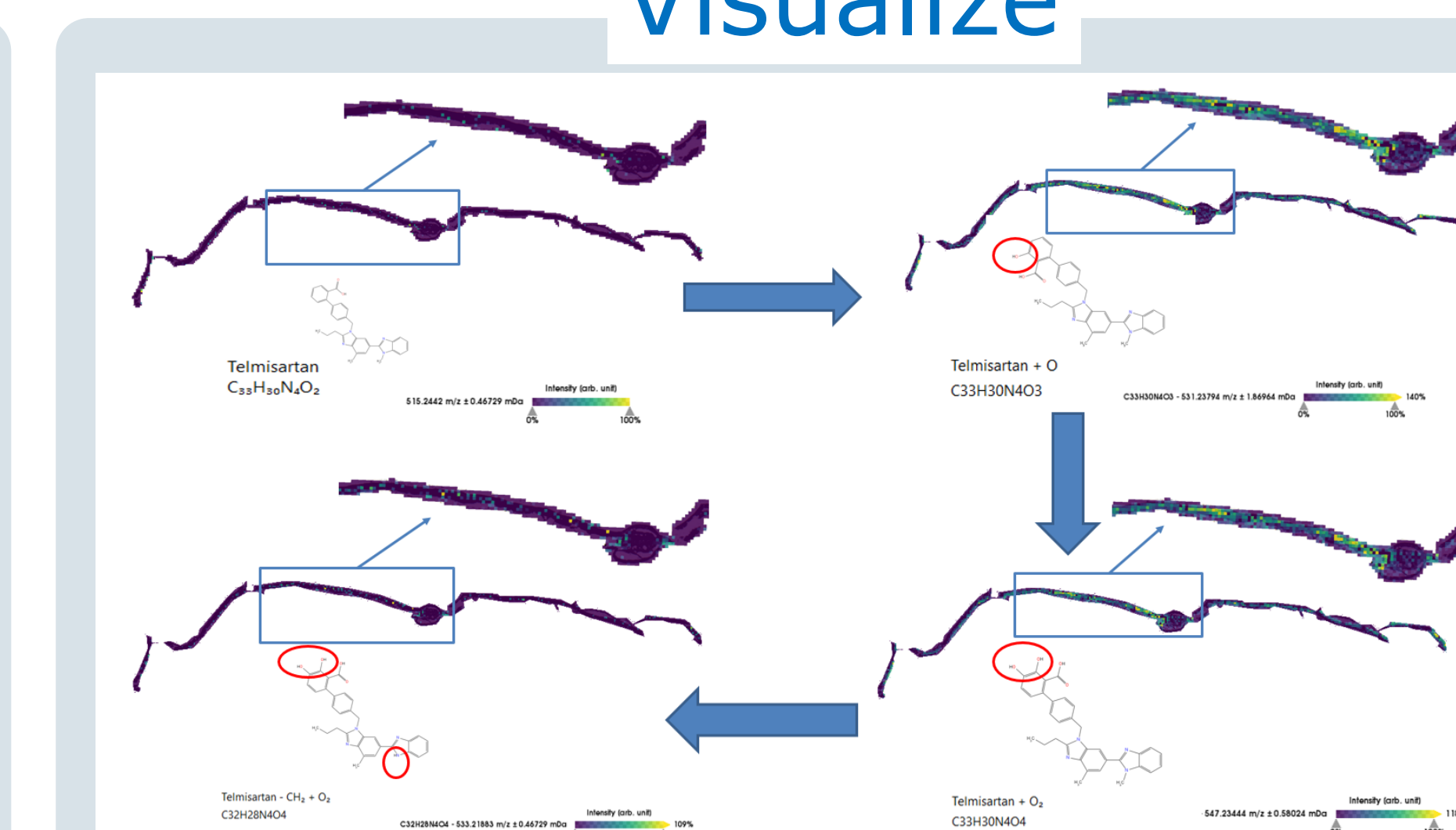
Visualize

Fig. 4 Tissue distribution of Telmisartan and its three metabolites in S. alba leaves. The developed workflow enabled to annotate and readily detect differences in spatial distribution for xenobiotic first and second generation metabolites (structures assignments are tentative).

Methods

White willow (Salix alba) leaves, as well as water and sludge samples were collected near the exit of a wastewater treatment facility. LC-MS analyses were conducted using an impact II QTOF system (Bruker) [2]. Mass spectrometry imaging was performed on the leaf tissues using matrix assisted laser desorption ionization (MALDI) on a SolariX XR 7T (Bruker). Datasets were visualized in SCiLS Lab 2020a to determine and define tissue regions of interest with differentiating m/z localizations. Regions of interest generated in SCiLS Lab 2020a were exported to a pre-release version of MetaboScape 2021 (Bruker) for compound annotation (Fig. 1). For xenobiotic metabolite prediction the BioTransformer* tool "SuperBio" rule set was triggered from within MetaboScape on a local server (see Fig. 2). For applying the "Environmental Microbial Transformation [3]"** rule set the BioTransformer online version was used. Generated SDF files enabled automatic assignment of xenobiotic metabolites (Fig. 3). These annotations were imported back to SCiLS Lab 2020a to study the tissue localization of the metabolites.

Results & Discussion

- By non-targeted LC-MS metabolome analysis of water, sludge and plant leaf extracts several xenobiotics including the drug Telmisartan could be annotated [1] (data not shown)
- To better understand the biological processes occurring in the white willow leaves exposed to Telmisartan and other xenobiotics, mass spectrometry imaging (MSI) was applied and datasets were investigated using an evaluation pipeline based on SCiLS Lab and MetaboScape softwares.
- Annotation of the dataset with BioTransformer predicted drug metabolites allowed for assignment of several xenobiotics
  - Annotation Quality Scoring enabled to quickly assess confidence for each automatic annotation (see Fig. 3)
  - Predicted Phase 1 and 2 metabolites and their reaction mechanisms were investigated by intuitive visualizations (see Fig. 2 and 3)
- Investigation of annotated m/z signals in SCiLS Lab revealed leaf specific tissue localizations for xenobiotic metabolites
  - Tissue specific localizations of predicted Telmisartan metabolites including phase one oxidized forms ($C_{33}H_{30}N_4O_3$) and second-generation metabolites ($C_{33}H_{30}N_4O_4$) were detected (see Fig. 4)

Summary

The presented MSI workflow proved useful and complementary to LC-MS based environmental metabolomics studies for investigating spatial distribution, metabolic effects and metabolism of xenobiotics in plants (here S. alba) chronically exposed to micropollutants in environmental conditions.

Conclusions

- A software pipeline for targeted exploration of metabolic processes directly on plant tissue exposed to xenobiotics is shown for the first time
- An integrated workflow based on SCiLS Lab and MetaboScape was applied for MSI data processing
- The BioTransformer* tool is triggered from within MetaboScape for local, i.e. secure, in-silico metabolism prediction
- BioTransformer based compound annotation was performed using m/z accuracy, isotope pattern fitting extracted by the T-ReX² algorithm
- The presented workflow enables assignment of the "dark matter" of the metabolome – molecules that remained uncharacterized before

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