New CCS Prediction Workflow to Extend MS/MS Spectral Libraries On The Fly with CCS Information

Sofie Weinkouff[1]; Nikolas Kessler[1]; Aiko Barsch[1]; Matthew Lewis[1]; Lucy Woods[1]; Heiko Neuweger[1], Bruker Daltonics GmbH & Co. KG, Fahrenheitstraße 4, 28359 Bremen, Germany

Introduction

The determination of the Collision Cross Section (CCS) has shown to increase the confidence in annotation and identification of small molecules in various ways.

In Trapped Ion Mobility Mass Spectrometry (TIMS) CCS values can be used as an additional, orthogonal qualifier for confident compound annotation in addition to mass accuracy, retention time fit, isotopic pattern fit and MS/MS spectra matching.

Still, publicly available CCS libraries are far away from covering the entire space of small molecule compounds.

The new workflow for automatic CCS prediction for any user-created or publicly available MS/MS libraries with structure information allows for CCS-enabled annotation even in the absence of reference CCS values.

Methods

Software Development:

- CCS-Predict, a machine learning CCS prediction tool in MetaboScape®, was integrated into automated MS/MS spectral library annotation.

Data acquisition:

- Human urine samples were measured with LC-TIMS-MS/MS using a timsTOF Pro 2 MS instrument (Bruker) in combination with a 23 minutes reversed phase UHPLC gradient.

Data processing and annotation:

- Processing and annotation was performed with a preliminary version of the MetaboScape 2023 software (Bruker).
- Untargeted compound annotation was performed using the automatic CCS prediction for spectral libraries in MetaboScape with the Bruker NIST 2020 MS/MS spectral library [1].
- To confirm selected predicted CCS values, annotations were additionally validated with retention times from Bruker HMDB Metabolite Library [2] and reference CCS values from the CCS Compendium [3].

Results

- Automated CCS prediction using CCS-Predict is fully integrated into the spectral library annotation workflow of MetaboScape (Fig. 1).
- The CCS-Predict Pro model was trained on data consisting of a broad range of small molecules structures and CCS references.

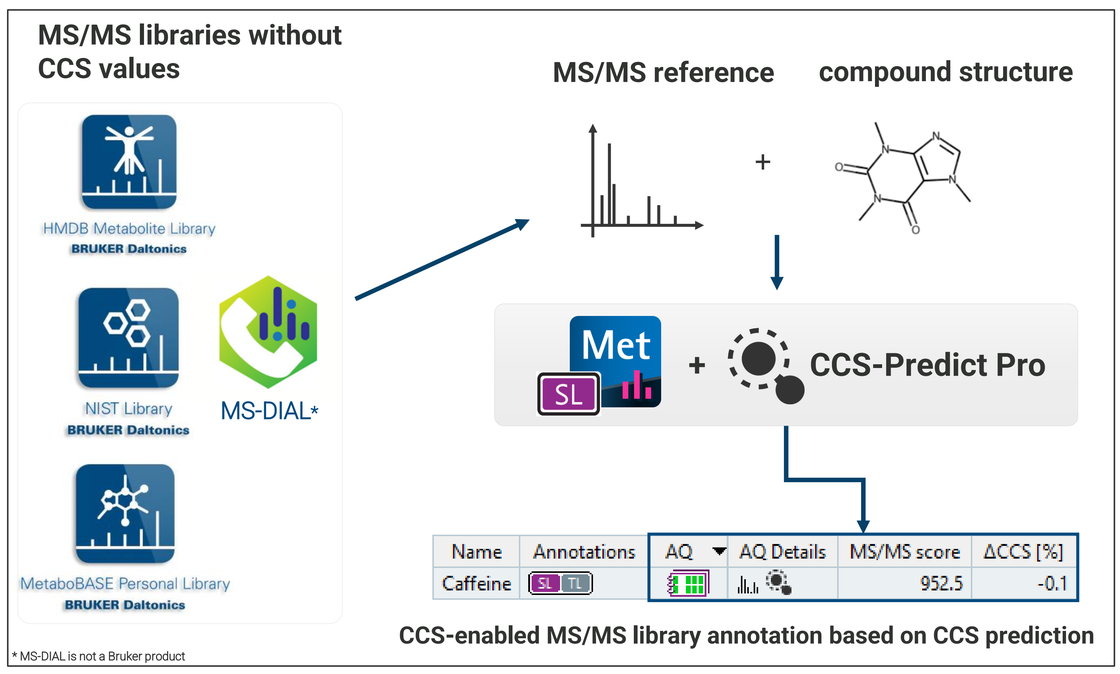

Fig. 1 The new CCS prediction for MS/MS spectral library annotation enables CCS-enabled annotation for any user-created or publicly available MS/MS library with structure information. Using the structure of a compound and the new CCS-Predict Pro machine learning model as a basis, CCS values are predicted and matched against measured CCS values for increased annotation confidence.

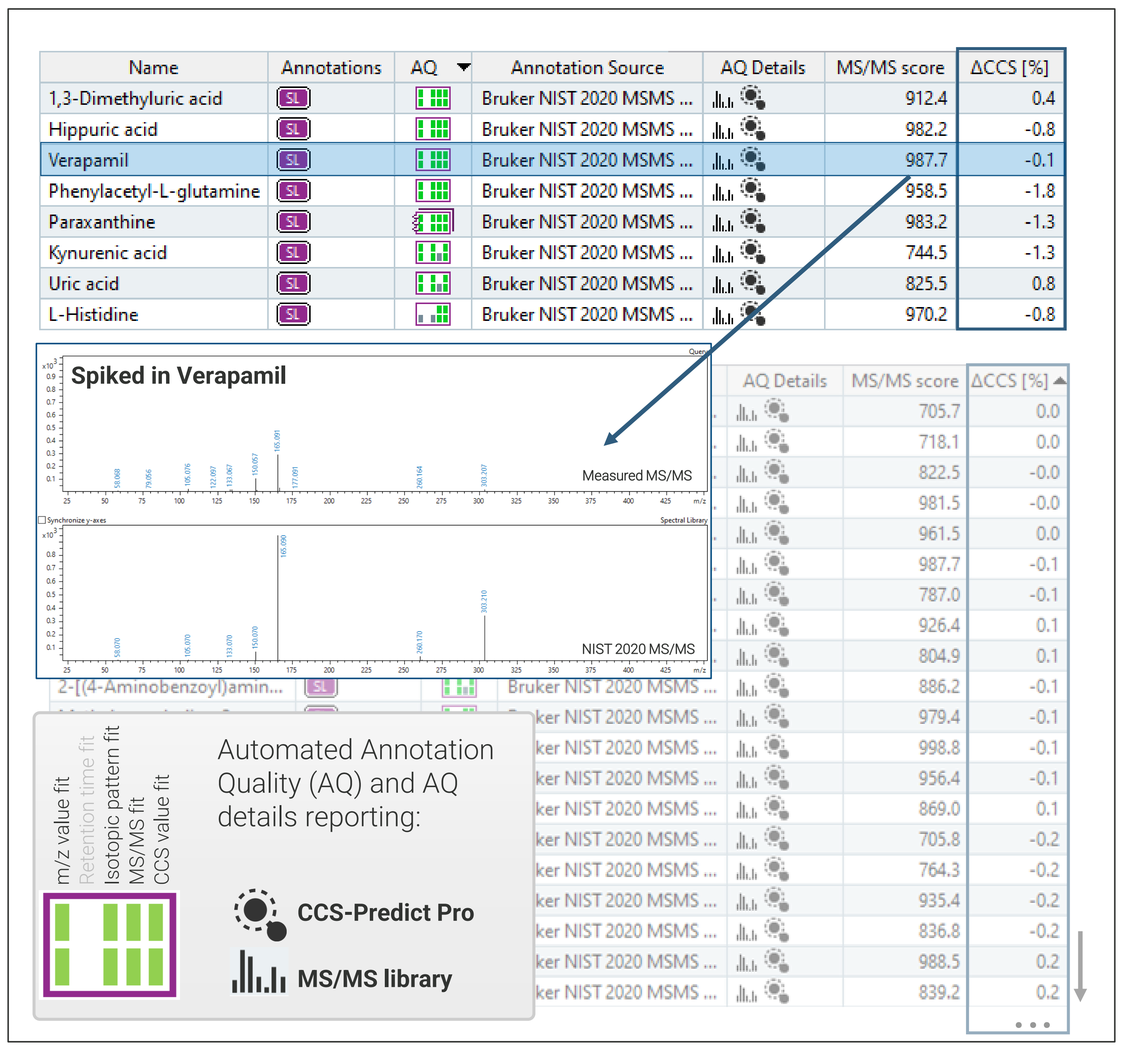

Fig. 2 The new CCS-Predict workflow increases confidence in annotations by providing deviations between measured and predicted CCS values as additional qualifiers for MS/MS spectral library-based compound annotation. Automated annotation quality and AQ details reporting helps to assess confidence and to filter the annotated features.

- Features were annotated using the NIST 2020 MS/MS library and filtered with automated AQ scoring in MetaboScape.
- 145 unique annotations with relative CCS deviations below 3 % (Fig. 2), which was recently reported as the acceptable deviation between measured and predicted CCS values by Das *et al.*, 2022 [4].
- 40 unique annotations with highest possible annotation confidence in all 4 criteria meaning mass dev. < 2 ppm, MS/MS score > 900, mSigma < 20, CCS deviations measured vs. prediction < 2 %.
- L-Histidine and Paraxanthine (tentative annotations) as well as spiked in Verapamil showed to be in line with reference retention times from HMDB and CCS references from CCS Compendium.
- Structures from the training data were used to calculate molecular descriptors (based on calculated physico-chemical properties).
- Molecular descriptors and reference CCS values were used to generate feature vectors that served as the basis for the new Support Vector Regression (SVR) model CCS-Predict Pro.
- Any MS/MS spectral library with structure information can be imported into MetaboScape and used as a CCS-enabled library.
- CCS-Predict is triggered automatically during spectral library annotation and first predicts a CCS value from provided structure information, second calculates a deviation between measured and predicted CCS and lastly assigns a CCS-based annotation confidence on-the fly, without modifying the original library.
- All annotations receive automatic Annotation Quality (AQ) scoring, AQ details and CCS deviation between measured and predicted.

References

[1] https://store.bruker.com/products/bruker-nist-mass-spectral-library
[2] https://www.bruker.com/en/products-and-solutions/mass-spectrometry/ms-software/metabolomics-spectral-libraries.html
[3] https://doi.org/10.1039/C8SC04396E
[4] https://doi.org/10.1021/jasms.1c00315

Summary

Here, we present a novel workflow for large-scale CCS-enabled MS/MS spectral library annotation using automated CCS prediction with CCS-Predict Pro.

Conclusion

- Novel annotation workflow combining automated CCS prediction with MS/MS spectral library annotation
- CCS-enabled annotation of any user-created or publicly available MS/MS library.
- Unlocks higher annotation confidence for existing MS/MS libraries even in the absence of CCS references.

4D-Metabolomics