INNOVATIVE METABOLOMICS APPROACHES FOR THE EFFICIENT SEARCH OF VALUABLE BIOACTIVE NATURAL PRODUCTS

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With the recent progresses made in metabolite profiling methods and miniaturization of bioassays, a question that arises is: do we still need to perform conventional large scale bioactive guided-isolation of natural products? All these rapid innovations may lead to a change of paradigm in natural products research towards more efficient and rational approaches in the field.

High resolution mass spectrometry (HRMS) and data dependent MS/MS analyses provide very valuable information on secondary metabolites for in-depth metabolome annotation studies.[1] The recent development of molecular network (MN) approaches for the mining of such data in combination with spectral database generated *in silico* [2] gives the possibility to establish relationships between metabolites thus significantly improving the efficiency of dereplication when combined with high quality chemotaxonomic data.[3] Such types of information can be generated with a few mg of extract only and are readily applicable to herbarium scale samples. In addition, such data can be statistically correlated with bioactivity data on extracts and potentially enable the localisation of interesting hits without the need for a bioactivity-guided approach.

For complete *de novo* identification of new compounds MS-targeted micro-isolation can be performed and sensitive 1D and 2D microNMR with microgram amounts of purified metabolites can be acquired. For bioactivity determination, many bioassay fit also to this scale. Using an ideal combination of methods it is this virtually possible to fully identify any bioactive principles in this way. Integration of other filters

to this approach such as permeation studies on extracts additionally provide key information on the possible bioavailability of NPs prior to their isolation. Furthermore the link of a given bioactivity result to those previously reported for compounds similar to those identified can be rationalised through *in silico* chemical space approaches.

Ideally a combination all these state-of-the-art methods should enable to identify and localise valuable NP efficiently at the analytical scale. In such a way large scale MS-targeted isolation of valuable NPs only can become a very rational way to conduct investigations. Different recent applications of our metabolomics and phytochemical investigations will illustrated these aspects.

A summary of what could be an ideal workflow will be presented and discussion on what is readily implemented and what is still required will be made.

[1] Wolfender J-L, Marti G, Thomas A, Bertrand S. Current approaches and challenges for the metabolite profiling of complex natural extracts. J Chromatogr A 2015 1382: 136-164.

[2] Allard PM, Peresse T, Bisson J, Gindro K, Marcourt L, Pham VC, Roussi F, Litaudon M, Wolfender JL. Integration of Molecular Networking and In-Silico MS/MS Fragmentation for Natural Products Dereplication. Anal. Chem. 2016 88: 3317-23.

[3] Allard P-M, Genta-Jouve G, Wolfender J-L. Deep metabolome annotation in natural products research: towards a virtuous cycle in metabolite identification. Curr. Opin. Chem. Biol. 2017 36: 40-49.