Strategies for molecular structure elucidation in static and dynamic systems

Valorization

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Creating impact has been the prime motivation and goal of my doctoral journey. Central was the creation of fundamental insight into principles of ion mobility spectrometry, new approaches in surface plasmon resonance, and even important the use of these technologies for in situ investigation of dynamic processes. The research described in this thesis, alongside other research and educational activities carried out during my PhD, was aimed at fulfilling this objective. As described below, some of the research outcomes had an immediate impact and some are anticipated to have a more long-term effect on academia, industry, and society.

Academic impact

One of the academic impacts, common to all chapters of this thesis, relates to the scientific breakthroughs in the field of drug discovery. Chapter 1 addresses this process from the small molecules' point of view. Chapter 1- Section 1 introduces an ion mobility-based methodology to discriminate structurally similar drug metabolites. The method is not exclusive to the test molecules, but rather a general approach applicable to a wide of range small molecules, enabling analytical chemists to implement a similar approach for the separation of isomeric compounds, which is necessary for pharmacokinetic and toxicological studies. In addition, this section discusses in detail the features of two types of ion mobility instruments. This can guide scientists to choose a suitable instrument for answering their research questions and improving their scientific impact.

Chapter 1- Section 2 evaluates a set of isomeric bile acids which are involved in a large variety of (patho)physiological conditions in the liver, kidney, intestine, lung, and even brain. Their diverse function on basis of small changes in their molecular structure, and therefore selective determination in biological materials, was the main rationale for studying their behavior in ion mobility spectrometry (IMS). The discussed science introduces different separation approaches by IMS and tandem mass spectrometry without chemically manipulating the molecules. The introduced methodology enables researchers to investigate the role and importance of structural isomers in the underlying molecular mechanisms of different diseases. These approaches were designed in a way to be translatable to complex biological samples, hence could be of use for clinical samples as well. In addition, this section aimed to fill one of the most important gaps in the field of IMS. Despite fast-growing progress in this field, there is limited knowledge on interpreting the actual mobility and collision cross section, while understanding the fate of molecules after ionization and throughout their trajectory in the mass spectrometer. This is essential for researchers to extract the correct information from their results and perform smart planning for (pre-) treatment of complex samples. The hyphenation of IMS with MS introduces the possibility of spatially resolving bile acids in a tissue by mass spectrometry-based imaging.

The scientific breakthroughs of Chapter 2 are related to the fast-developing area of native protein analysis. Chapter 2- Section 1 introduces a rediscovered buffer for studying proteins in their natural-like form by mass spectrometry. This opens a door for researchers to analyze a diverse range of proteins for target or biomarker identification

as well as structural and kinetic characterizations in relation to their biological activities. In addition, it provides the scientist with the possibility to elucidate molecular mechanisms involved in (phato)physiological conditions.

The initial results and observations from the previous section sparked the idea to further investigate the interaction of cardiac protein troponin subunits with surface plasmon resonance (SPR). For the SPR research project described in section 2, I was granted the competitive researcher fund of SWOL (University Fund Limburg). This section serves two main scientific impacts. One is suggesting a new approach for investigating the interaction kinetics of a complex protein's subunits. The other one is an attempt to study the kinetics of involved proteins in myocardial infarction. Myocardial infarction (MI) is one of the leading causes of death. The presented approach could ultimately unveil the proteins interaction mechanism, which can eventually aid in designing efficient diagnostic methods for discriminating MI from non-MI.

Chapter 3 is mainly focused on hyphenation sciences for studying small and large molecules. Chapter 3- Section 1 is a critical review of applied strategies to connect organ-on-a-chip with mass spectrometry. With this review, researchers can not only get an overview of previous attempts and discoveries but also, they can realize the shortcomings and focus on solving them.

The presented work in section 2 is adhering to the highly desired need for "flow chemistry", anticipated to be a more green and sustainable approach in the production of high-quality (purity) chemicals. The application of the specific design MS/MS -IMS scanning strategies and the use of fundamental insight into tandem mass spectrometry processes, allowed the in-situ detection of structural isomers, with even highly comparable MS/MS spectra. IMS served as a pre-separation step, before unraveling the structures by MS/MS. This approach is of special interest in the area of "Process Analytical technologies" as it enables chemists in a large variety of industrial applications to monitor chemical conversing processes, with high sensitivity and molecular structure resolved. The latter not being possible with the current and frequently applied IR and NMR technologies.

The majority of the findings and results presented in this thesis are published in open and peer-reviewed scientific journals. Hence, researchers with different scientific backgrounds can easily access the presented science and translate it to their research questions. The immediate academic impact of the published work in chapter 1- section 1 is reflected in the recognition by the journal of rapid communication in mass spectrometry (RCM). This article was recognized as one of the most read and downloaded papers in this journal between 2018 and 2019.

The PhD journey would not be complete without an impact on educating the next generation of scientists. Besides lecturing and tutoring that I carried out, one of the most effective approaches in transferring hard and soft professional skills as well as knowledge is to supervise undergraduates. The immediate academic impact of supervising 12 students (including HBO, bachelor, and master students) is reflected in their successful advancement in their following careers and education. In addition to

supervising students in STEM sciences, supervising honours+ students from social, political, and economic sciences added another dimension to the academic impact of my journey.

Industrial Impact

During my PhD journey, I had the unique opportunity to collaborate with multiple industrial partners active in the fields of polymer materials (DSM Materials Research Center), production of industrial enzymes (DSM Food), advanced chemical synthesis and biocatalysis (Innosyn), drug delivery and click chemistry (Cristal Therapeutics), pharmaceutical R&D (Janssen Pharmaceutica), synthetic biobased polymers (Be4Plastics), producer of flow-reactors (Chemtrix) and the manufacturer of advanced SPR technologies (Bionavis).

For instance, the performed projects together with DSM centers were focused on separation sciences for fast and effective analysis of low caloric sweeteners (steviol glycosides) and metabolites of a drug candidate for epilepsy disease. The results of these fruitful collaborations were a published paper (Chapter 1- Section 1) as well as the implementation of the introduced principle in their routing analysis.

The performed projects in collaboration with Janssen Pharmaceutica led to three different publications including Chapter 1 – Section 1, a just-published paper in RCM as the shared co-first author as well as a published paper in the journal of Analytical and bioanalytical chemistry. My contribution to the latest paper was to develop a novel 3D-printed mold for quantitative mass spectrometry imaging. This mold is now routinely used in the laboratories of Janssen Pharmaceutica.

The fruitful collaboration with Cristal Therapeutics resulted in a publication in the journal of Chemical science and the granting of a patent for the specific click chemistry discovered. The patented CliCr technology has been recently sold to Synaffix. In the project, MS was applied for the in-situ monitoring of cyclo addition reactions (Click Chemistry), once again proving the ability of MS and possibly IMS technologies for the accurate monitoring of (bio)chemical conversions. The industrial impact of the in-situ MS experimentation is eminent.

Industry 4.0 or the fourth industrial revolution centers around smart factories, where continuous manufacturing processes such as "flow-chemistry" will play a dominant role in the further electrification and green production of the chemical industry. The implementation of flow chemistry and online reaction monitoring are two of the important drivers toward Industry 4.0. With this goal in mind, and in collaboration with Chemtrix, a commercially available microflow reactor was hyphenated with IMS-MS for the online reaction monitoring by IMS and MS/MS. This work (chapter 3- Section 2) was published in the journal of Flow chemistry to reach out to academic and industrial researchers proving the tremendous potential of the discussed and combined IMS, MS/MS strategies.

The success of our collaboration with BioNavis is reflected in the presented work in Chapter 2- Section 2. Our fruitful collaboration ensured that the SPR community is

familiarised with the unique feature of BioNavis for easy monitoring of the full angel range, routine analysis, and easy implementation in the industry. In addition, the introduced approach provides insights for pharma R&D to develop novel diagnostic strategies to be able to discriminate myocardial infarction from other diseases.

Furthermore, in the pursuit of translating fundamental research to functional products and solutions, together with NWO (Dutch Research Council) and Lorentz center, we organised the "Life Sciences with Industry" workshops in 2020 and 2021. This workshop encouraged cooperation and the exchange of knowledge between academia and industry. In a stimulating setting, scientists and researchers from both academia and industry worked closely together to find innovative solutions to scientifically challenging and commercially important R&D problems of the invited industry partners. Participants were young and talented scientists (PhDs, postdocs, and more experienced researchers) from different sectors of the life sciences and physics, guided by senior researchers from industry and academia. Even though we had to cancel our workshop in 2020 due to Corona restrictions, the success of this workshop in 2021 allowed me and others to directly benefit from the impact of scientific research in the context of business and industry.

Societal impact

The research projects of which a considerable part forms the basis of this thesis were directed to a more fundamental understanding of analytical technologies, the design of novel approaches to detect selectively structural isomers, and research (bio) chemical processes including click chemistry, biocatalysis and the dynamic interaction of protein subunits in the formation of 3D structures. These projects were performed in collaboration and co-creation with both academic and industrial partners from M4i, Maastricht University, Brightlands Chemelot R&D campus, and abroad. Besides a diversity of small molecules, proteins, and synthetic polymers were investigated broadening my scope on sciences. Hence, an additional target group of this thesis was improving the life quality of society, which includes not only patients but also their families, caregivers, and the social security system. The offered approach in this thesis will affect society from different aspects of diagnosis, prevention, treatment, and economy. Chapter 1- Section 1 is examining the metabolites of a potential drug candidate, targeting the treatment of patients suffering from epilepsy disease. Chapter 1- Section 2 is analyzing bile acids that are involved in multiple pathological pathways including neurodegenerative diseases such as Alzheimer's and Parkinson's. This study can potentially serve diagnostic, prevention, and treatment purposes. The same applies to Chapter 2- Section 1. This study is introducing an approach to analyze and study proteins as key elements for identifying biomarkers or targets. Chapter 2- Section 2 is directly analyzing cardiac troponin complex for elucidation of the mechanism of myocardial infarction to eventually come up with a more efficient diagnostic method. Chapter 3- Section 1 is an opening towards constructing a more precise platform for understanding disease mechanisms and finding the most optimal treatment approaches, particularly personalized medicine. Chapter 3- Section 2 is in line with the call of the FDA (Food and Drug Administration) to switch from batch process to continuous flow chemistry. The presented work offers an efficient approach to drug

discovery that can eventually increase the speed of medicine available to patients, reduce waste to provide an environmentally clean production method, and use less material to alleviate economic burdens.

In addition, as scientists, we are also responsible to communicate our contemporary scientific developments to society. This way we can keep them well informed and aware of our findings and future steps, receive their insight and hear their discussions and needs. To this end, I used two platforms TEDx Maastricht (2018) and Pint of Science Maastricht (2022) to engage the public with my PhD research and findings.

Lastly, my interest in the socio-political topic of inequality led me to participate in the "Honours+ program challenge" organized by Maastricht University EDLAB in 2020-2021 and 2021-2022. The results of these works are the publicly available critical literature studies with the proposed solutions for two specific inequality problems. The immediate and futuristic academic and societal impacts of these efforts could be valorized by winning the "Honours+ program challenge" for two consecutive years.