Scientific report 2010-2014

In 2010-2014 structural mass spectrometry subgroup work included mainly the chemical cross-linking and hydrogen/deuterium exchange (HDX) mass spectrometry. In both these fields, software tools allowing data interpretation and visualization were developed. This resulted in one publication describing web based MSTools [1] and creation of two programs – Xlynx and DeutEx (http://ms.biomed.cas.cz/SWD/). Both program packages are actively used by the team members and significantly increased the data processing speed. Other methodological development led to setup of a unique method for non-ionic detergent removal [2], which further allowed for structural study of a true membrane protein, ADP/ATP transporter [3]. While the first publication was mostly done in our lab (90%), the second one was mainly conducted by the collaborating laboratory in France. Petr Man participated at all stages of the project. Further methodological development focused on novel aspartic proteases suitable for protein digestion in HDX MS. We focused on proteases from carnivorous plants *Nepenthes*. First we described extremely efficient and simple protocol for preparation of large quantities on Nepenthesin-1 [4]. This protocol is also a part of US patent application, where the diagnostic use of these proteases is claimed - *US20140186330 A1, Treatment of gluten intolerance and related conditions* (with Petr Man and Hynek Mrazek as co-inventors). In a subsequent study we showed that Nepenthesin-1 is good alternative to commonly used pepsin in HDX MS protocol and the work also provided some general observation about immobilized protease columns and the use of denaturing agents during the digestion [5].

The application of chemical cross-linking and HDX MS was used to study solution structure on NK cell receptor CD161 isoform A and showed surprising differences between the crystal structure and solution structure [6]. In a subsequent study another combination of structural mass spectrometry techniques (cross-linking and ion mobility), in combination with molecular modelling were used to characterize the structure of NKR-P1C molecule in solution [7]. Combination of structural MS techniques was also applied in other studies either from our group [8-10] or from the collaborating laboratories we instrumentally and mentally supported [11-17].

In the field of protein glycosylation the group identified novel protein modification S-glycosylation (collaboration with Massey University, New Zealand, [18]) and studied the role of glycosylation in liver disease in collaboration with Georgetown University [19-21]. In addition to these main areas group members also took part in various proteomic studies [22-24] as well as in projects focused on secondary fungal metabolites [25,26].

The applied research is illustrated by development of ***Method of Surface Modification for the Purpose of Enrichment of Phosphorylated Peptides for Analysis by Desorption/ionization Mass Spectrometry Techniques***. The Czech patent has already been issued in 2012 (No. 303056) and we recently also received positive position from USPTO. The patent application described the construction of a spraying deposition device for the preparation of functionalized surfaces for laser desorption mass spectrometry [27].

Significant results have also been achieved in the field of **biomolecular imaging mass spectrometry** (IMS). The subgroup started with IMS in the Czech

Republic in 2009 when imaging ambient techniques were assembled on our previous FTICR mass spectrometer [28]. We soon switched our attention to nanostructure-assisted laser desorption ionization (NALDI) based on silicon nanowires [29] distributed by Bruker Daltonics (Bremen, Germany) as a matrix-free alternative to MALDI for analysis of small molecules [30]. The silicon nanowire surface was further oxidized and modified with (pentafluorophenyl)-propyldimethylchlorosilane and has gained an immediate analytical reputation. The surfaces soon were identified as useful DESI substrates [31]. Desorption nanoelectrospray (nanoDESI) was developed at Palacky University in collaboration with us [32]. The source was applied in chiral discrimination [33] or anthocyanin analysis [34] and recently technically improved and characterized in detail [35]. In our previous work also the murine tissue sections were imprinted to NALDI targets, tissues and salts washed out and lithographic transfers then visualized in microprobe scanning mode [36]. NALDI imaging was found faster than MALDI IMS due to the absence of the time-consuming matrix deposition step and NALDI images were cleaner as cationization effects were reduced in individual spots. In a more recent study the catalytic oxidative properties of NALDI surfaces were disclosed enabling double bond(s) localization in lipids and unusual peptides [37]. In our most recent work we disclosed the distribution of globotriaosylceramides in Fabry renal murine tissue [38] and dissected lipid degradation processes in human eye lenses [39]. We also described and rationalized the putative surface enhanced mass spectrometry effect with our newer SolariX FTMS system [40]. The head of laboratory has actively participated in EU COST action BM1104 (Mass Spectrometry Imaging: New Tools for Healthcare Research) as a Czech national representative. The collaborative EU work and gained knowledge in state-of-the-art imaging mass spectrometry [41] represents a basis for future expanding into multimodal imaging field.

Nuclear magnetic resonance group does a lot of contractual work (*see the 3-9 form*) but also carries out its own research. The group is engaged with applications of NMR to study larger **biomolecules as well as metabolomics**. An important part of the research activities is represented by **monitoring asymmetric reactions in organic synthesis**. In this project, we collaborate with the Institute of Chemical Technology in Prague. Together, we have a high number of publications in the years 2010 – 2014 concerning the asymmetric transfer hydrogenation of imines [42-55]. We developed a method to follow organic catalytic reactions in NMR tube enabling *in-situ* monitoring [49,51]. Special attention was paid to the study of the mechanism of catalytic hydrogenation using ruthenium catalysts of Noyori's type [44,47,53]. It was shown that the role of base during the reaction is more important than generally assumed. The results suggested that the protonated base formed an associate with the active ruthenium-hydride species, most probably via a hydrogen bond with the sulfonyl group of the complex. It is assumed that the steric and electronic differences among the bases were responsible for the observations monitored by NMR as well as FTICR MS [44].

Structure determination of **microbial secondary metabolites** represents another important part of NMR research activities. New substances like Puwainaphycins F and G isolated from the soil cyanobacterium *Cylindrospermum alatosporum* C24/89 were isolated and characterized [56]. Aeruginosin-865 from terrestrial cyanobacterium *Nostoc* sp. Lukesova 30/93 was also discovered. It poses quite a unique structure: it is the first aeruginosin-type peptide containing

both fatty acid and carbohydrate moiety. It is the first aeruginosin found in the genus *Nostoc.* It shows the anti-inflammatory activity without cytotoxic or barrier disruption effect [57].

Electron microscopy group operated rather obsolete equipment in period 2010-2014. On the contrary, this period was characterized by collaborative research supporting the whole biological campus in Krc [58-63]. The EM group followed two basic directions. The first one represented the characterization of the macromolecular complexes by classical electron microscopy. We used negative staining methods for characterization of recombinant human ameloblastin (AMBN), an intrinsically disordered matrix protein. Electron microscopy revealed that the AMBN high molecular mass self-assemblies were flat ribbon-like supramolecular structures with an average width of 18 ± 4 nm and with a variable size, ranging from tens to hundreds of nanometers in length. Further it was shown that N-terminal of the AMBN is involved in forming macromolecular structures and not the C-terminal [64]. Using similar methodological approach, the *Arabidopsis* Nitrilase-1 (NIT1) has been studied. We have shown that NIT1 in *Arabidopsis* is present in high-molecular-mass polymers and forms filamentous structures similar to bacterial and fungal nitrilases [59]. In the methodological approach we employed here an immuno-detection of NIT1 by specific antibodies coupled to colloidal gold. This allowed us to clearly detect NIT1 polymers and distinct them from other filamentous proteins, mainly of cytoskeletal origin, which were also presented in the system.

The second direction was characterized as studies of biological surfaces. In this relatively broad scope we dealt with the interactions of intestinal bacteria and intestinal mucosa, especially gliadin affected surfaces. The classical scanning electron microscopy was used through the study [65]. The complex electron microscopic approach (TEM + SEM) was employed in the study of *Streptococcus pneumoniae* LocZ mutants morphology and ultrastructure with regard to the cell division process [66]. We showed that Z-ring formation and placement is strongly affected by mutations in the LocZ.

Luckily, we acquired a new SEM in late 2014 opening new laboratory possibilities in sample handling and our own development work. The new FEI Nova NanoSEM 450 is a field-emission gun equipped high resolution **scanning electron microscope** with SE, TLD detectors for secondary electrons, CBS detector for back-scattered electrons and EDAX Octan plus detector for EDS microanalysis.

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