Annex no 4

Summary of the Professional Scientific Achievements

Irena Đapić

International Centre for Cancer Vaccine Science, University of Gdańsk

Summary of the Professional Scientific Achievements

Development of tools based on mass spectrometry for proteomic analysis of biological materials

Dr Irena Đapić

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Gdańsk 2022

1. Name: **Irena Đapić**

2. Awarded Diplomas and Degrees (Institution, Department/Faculty or any other Research Unit, Date of obtaining Academic Degree, title of the PhD thesis)

2014 PhD, Department of Chemistry, Faculty of Science in Zagreb, Zagreb, Croatia (Title: „Development and validation of the biochemical indicators of the skin barrier function". Supervisors: Prof. Ivone Jakasa, PhD and Renata Kobetic, PhD (PhD awarded on 14/11/2014)

2008 MSc in chemistry, Department of chemistry, Faculty of Science, University of Zagreb (Title: „Adsorption of bovine serum albumin on previously formed polyelectrolyte multilayer"). Supervisor: Prof Davor Kovacevic, PhD (MSc diploma awarded on 17/12/2008)

3. Information on previous employment in Scientific Institutions

July 2018-	Principal investigator (Chemical Biology Group Leader), International Centre for Cancer Vaccine Science, University of Gdańsk, Gdańsk, Poland
2017-July 2018	Postdoctoral researcher in Laboratory for Analytical Chemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia
2015-2017	Postdoctoral researcher in Biomolecular Systems Analytics group, van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, the Netherlands
2014-2017	Postdoctoral researcher in Laboratory for Analytical Chemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia
2009-2014	Scientific and Teaching Assistant in Laboratory for Analytical Chemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

4.1. Title of the scientific achievement:

Development of tools based on mass spectrometry for proteomic analysis of biological materials

4.2. Research publications belonging to the Scientific Achievement:

IF – impact factor from the year of publication of the work; if IF was not present for the specific year, the IF of previous year was used; $IF_{5\text{-year}}$ – 5-year impact factor; MNiSW journal scoring according to Ministry of Science and Higher Education: citations (Web of Science (WoS: citations/without self-citations); Google Scholar)

1. **Dapic, I.***; Uwugiaren, N.; Kers, J.; Mohammed, Y;. Goodlett, D.R.; Corthals, G.* Evaluation of Fast and Sensitive Proteome Profiling of FF and FFPE Kidney Patient Tissues. Molecules 2022, 1137
   IF= 4.412; $IF_{5\text{-year}}$= 4.558; MNiSW= 100 pkt; citations (WoS: 0/0, Google scholar: 1)

2. Pirog, A.; Faktor, J.; Urban-Wojciuk, Z.; Kote, S.; Chruściel, E.; Arcimowicz, Ł.; Marek-Trzonkowska, N.; Vojtesek, B.; Hupp, T. R.; Al Shboul, S.; Brennan, PM; Smoleński, RT; Goodlett,

DR; **Dapic, I.*** Comparison of Different Digestion Methods for Proteomic Analysis of Isolated Cells and FFPE Tissue Samples. Talanta 2021, 233, 122568.
IF= 6.057; $IF_{5\text{-}year}$= 5.386; MNiSW= 100 pkt; citations (WoS: 6/4, Google scholar: 6)

3. Weke, K.; Singh, A.; Uwugiaren, N.; Alfaro, J. A.; Wang, T.; Hupp, T. R.; O'Neill, J. R.; Vojtesek, B.; Goodlett, D. R.*; Williams, S. M.; Zhou, M; Kelly, RT; Zhu, Y; **Dapic, I.*** MicroPOTS Analysis of Barrett's Esophageal Cell Line Models Identifies Proteomic Changes after Physiologic and Radiation Stress. Journal of Proteome Research 2021, 20, 2195-2205
IF= 4.466; $IF_{5\text{-}year}$= 4.352; MNiSW= 100 pkt; citations (WoS: 1/0, Google scholar: 1)

4. **Dapic, I.***; Baljeu-Neuman, L.; Uwugiaren, N.; Kers, J.; Goodlett, D. R.; Corthals, G. L.* Proteome Analysis of Tissues by Mass Spectrometry. Mass Spectrometry Reviews 2019, 38, 1–39.
IF= 8.887; $IF_{5\text{-}year}$= 8.479; MNiSW= 140 pkt; citations (WoS: 16/14, Google scholar: 22)

5. **Dapic, I.**; Uwugiaren, N.; Jansen, P. J.; Corthals, G. L. Fast and Simple Protocols for Mass Spectrometry-Based Proteomics of Small Fresh Frozen Uterine Tissue Sections. Analytical Chemistry 2017, 10769–10775.
IF= 6.042; $IF_{5\text{-}year}$= 6.035; MNiSW= 140 pkt; citations (WoS: 14/10, Google scholar: 19)

6. Wouters, B. †; **Dapic, I.** †; Valkenburg, T. S. E.; Wouters, S.; Niezen, L.; Eeltink, S.; Corthals, G. L.; Schoenmakers, P. J. A Cyclic-Olefin-Copolymer Microfluidic Immobilized-Enzyme Reactor for Rapid Digestion of Proteins from Dried Blood Spots. Journal of Chromatography A 2017, 36–42.
IF= 3.716; $IF_{5\text{-}year}$= 3.713; MNiSW=100 pkt; citations (WoS: 17/16, Google scholar: 25)

† *equal contribution*
* *corresponding or co-corresponding author*

Summarized IF of publications belonging to the scientific achievement = 33.580
Summarized $IF_{5\text{-}year}$ of publications belonging to the scientific achievement = 32.523
Summarized value of the Ministerial Publication Points for publications belonging to the scientific achievement = 680

4.3. Description of the research aims and results of the above-mentioned Scientific Achievement along with a description of their potential applications

Introduction

Analysis of human tissues and biofluids respresents an important source of molecular information of patient's status or their response to treatment. Proteomics has proved to be an important tool in such research as many proteins are directly involved in tumourgenesis and as such can be used as condition indicators. Cancer is a dynamic disease in which environment plays an important role and during tumour growth they become more heterogeneous and develop cells with different molecular signatures and distinct sensitivities to treatment.[1] Tumour heterogeneity results in development of genetically distinct cell-subpopulations that require development of sensitive and reliable tools for proper assessment.

Today, mass spectrometry (MS) has become an indispensable tool in the discovery phase of proteomics.[2] Limited amounts of a sample are often the norm when studying tumours or profiling cellular subsets from tumour microenvironment[3–5] or circulating tumour cells with the aim to obtain information to be used to follow a patient's status or response to therapy.[6–8] Continuous advances in MS instrumentation development also aim to increase instrumental sensitivity and deliver faster and reliable measurements.

Data dependent acquistion (DDA) has for a long time been the most popular way of data acquisition, however stochastic sampling of the most intense ions has often caused low proteome coverage and reproducibility; an important parameter when estimating methods reliability. Therefore, in the last years more attention has been given to data independent acquisition (DIA) which showed higher reproducibility in protein quantitation, and is of particular interest to study human tissues. DIA has also been used to obtain „digital maps" of the patient samples, which can be used repeatedly to test *in silico* for new biochemical hypotheses or clinically relevant biomarkers.[9]

Human tissues are used in proteomic studies as a source to reveal insight for the discovery of biomarkers or therapeutic targets. After surgical removal tissues are usually used for histopathological observations or tissues are preserved for further analysis. Tissues are mostly preserved as formalin-fixed paraffin-embedded (FFPE) or fresh frozen (FF) tissues. FFPE tissues are stored at room temperature and have been shown to be stable over long periods of time. Even though FFPE tissues present lower storage costs compared to FF tissues, it is known that formalin fixation can cause chemical modifications and cross-linking between proteins therein. An example of such a molecular formaldehyde-induced modification occurs on lysine as methylation (+14 Da), methylene (+12 Da) and methylol (+30 Da) modifications. Other modifications that might occur are intra- and inter-chain methylene bridges with amine, amide, guanidyl, phenol, imidazole, and indole groups of amino acids residues.[10,11] Studies that used paired FFPE and FF tissues to compare molecular information and overlap of diagnostically relevant proteins found that the subset of detected proteins varied by extraction method, tissue type and the instrument used for proteins detection. [11,12] Lysine side chains are mostly involved in a reaction with formaldehyde leading many studies to report lysine to arginine ratio (K/R) as a way to demonstrate the level of chemical modifications in FFPE tissues.[13]

To avoid chemical modifications of proteins induced by fixation process, researchers often use FF tissues. Preservation of FF tissues is simpler and faster than FFPE tissue preservation methods, and freezing has been regulated since 2006 by the European Human Frozen Tumor Tissue Bank (TuBaFrost) with regard to collection, storage, retrieval, and tracking of tissues.[14] An advantage of this type of preservation by freezing is that it avoids molecular changes induced by chemical modifications of proteins.[15,16] Frozen tissues are stored at low temperatures (−80°C) or in liquid nitrogen-cooled Dewar flasks (−196°C). Proteins are usually extracted using extraction buffers such as ammonium bicarbonate, Tris/TrisHCl or RIPA buffer that are often supplemented with detergents, organic solvents and chaotropes to improve protein unfolding and presentation to enzyme.[17,18] Extraction buffers often contain detergents to improve protein solubilization. Sodium dodecyl sulfate (SDS) has traditionaly been used for protein extraction, however, due to incompatibility with MS analysis it is not always appropriate for use and in some studies has been replaced with MS-compatible detergents such as Rapigest[19,20], MasDes[21] or Invitrosol.[22]

Development of technologies for microscale analysis is important when it is necessary to profile cellular subsets or tissue regions from a limited amount of sample. In recent years there has been an increase in the number of technologies developed to analyze proteins from limited amounts of the cells. Some methods such as nanoPOTS and Microdroplet Processing in One pot for Trace Samples (microPOTS) have been developed to use miniaturized sample preparation for in-depth analysis of proteomes.[23,24] These methods use a one-pot strategy where the whole sample preparation procedure is carried out in low-microliter or nano-liter droplet-based vessel. To further avoid adsorptive loss, all sample preparation chemistry is carried out in one location (i.e. well or vessel) thus minimizing protein losses during preparation that occur during traditional methods where the sample is moved frequently during preparation. This method was successful for analysis of less than 100 proteins, which is of particular

interest for profiling of patient derived biopsies or samples which have been excised with laser capture microdissection (LCM).[25] Moreover, technologies such as immobilized enzyme reactors (IMERs)[26–28] compared to in-solution sample processing offer a number of advantages starting with the fact that the whole procedure is performed in a small confined volume allowing higher enzyme to protein ratio, and also preventing autodigestion. Enzymes and proteins are closer in space to each other which shortens digestion time and typically immobilized enzymes are more stable compared to the same enzymes used in solution. Their advantage is that they can create integrated systems with MS instrument and IMERs require only small amounts of sample for processing, thus making them suitable for clinical applications.

Summary of the most important achievements:

The main goal of this scientific achievement is to demonstrate my work in development and evaluation of technologies and approaches for accurate, reliable and robust analysis of small amounts of human materials (tissues and dried-blood-spots, DBS). In my research I have used mass spectrometry (MS) as a tool to investigate proteomic signatures from limited amounts of material. I have particularly kept in mind limitations that come as instrumental loss of sensitivity and adsorptive losses of the materials when limited number of cells are analyzed. Therefore the most appropriate materials for development of technologies that would facilitate protein digestion (IMER) were used. Moreover, when examining appropriatness of FFPE tissues for proteomic analysis I have been led by clinical practice and in collaboration with pathological departments used appropriate (small) amounts of human tissues (microdissected tissues, biopsies). In the collaboration with Leiden University Medical Centre (LUMC), Amsterdam Medical Centre (AMC) and Centre for Clinical Brain Sciences, University of Edinburgh (UoE), I obtained different types of the human materials, which were further screened for proteomic signatures.

I have investigated also technological (instrumental) and chemical approaches to facilitate protein extraction and digestion. Particular attention was given to chemical modifications of the proteins and peptides that can emerge during sample preparation proceduers (i.e. carbamylation with urea) or the chemical modifications which arise from tissue storage (FFPE tissues). Moreover, I have investigated minimal amount of microdissected FF tissues that would be neccessary to obtain reliable information from human uterine tissue. Usage of technologies such as pressure cycling technology (PCT, Barocycler), nanoPOTS and microPOTS gave the advantage to analyze limited amounts of the cells. This could further be of particular importance when analyzing samples from patients and profiling different regions of tumours.

1. Proteome profiling of small tissue amounts

In the study of microdisected FF and FFPE human tissues I investigated several protocols that were shown to be sensitive and reliable for protein extraction and detection with LC-MS. Here I have also shown appropriatness of biopsy size of kidney FFPE tissues for proteomic research and have directly compared it with FF tissues. MS-compatible detergents were used for protein extraction and they showed their appropriatness in extraction of proteins from limited amounts of material. Moreover the effect of urea-induced carbamylation under elevated temperature and its influence on modification of lysine and arginine resiudes was investigated.

2. Development and application of technology for rapid protein digestion

To facilitate protein digestion and significantly shorten overall analysis time of the samples cyclic-olefin-copolymer (COC) microfluidic reactor containing trypsin immobilized on a polymer monolithic material via azlactone chemistry was developed. IMER was successfully used for digestion of proteins

from dried blood spots (DBS). Overall sample analysis time was several times shorter compared to traditional in-solution overnight digestion. IMER showed an advantage over in-solution digestion and in further work it could be coupled in-line with LC system which would allow high-throughput of the samples.

3. Proteome profiling of a limited number of cells

MicroPOTS technology was used to take advantage of one-pot sample preparation of limited number of the cells. Applicability of microPOTS system was shown for proteomic screening of approximately 200 cells with high reproducibility of the measurements. Importance of the results is in the further use of such microscale technologies to analyze directly patient samples of limited amount. This is of particular interest in the analysis of tumoural regions or tissue-derived biopsies.

Detailed description of the publications included in the Scientific achievement:

1. **Dapic, I.***; Uwugiaren, N.; Kers, J.; Mohammed, Y;. Goodlett, D.R.; Corthals, G.* Evaluation of Fast and Sensitive Proteome Profiling of FF and FFPE Kidney Patient Tissues. Molecules 2022, 1137
   IF= 4.412; $IF_{5\text{-}year}$= 4.558; MNiSW= 100 pkt; citations (WoS: 0/0, Google scholar: 1)

Aim of the study:

In this study I investigated biopsy sized FFPE kidney tissues and evaluated their appropriatness for use in clinical proteomics. Results obtained were compared with paired FF tissues that are usually stored at −80 °C and do not undergo chemical modifications as FFPE tissues. FFPE tissues as important clinical material are used for pathological assesment of patient's condition, however, during their preservation formaldehyde induced cross-linking within the tissue and protein matrices occur. These alternations in the structure and chemical composition of proteins often leads to poor chromatographic performance, shifting of retention times and missed identification of proteins. This is even more exacerbrated when small amounts such as tissue biopsies are used.

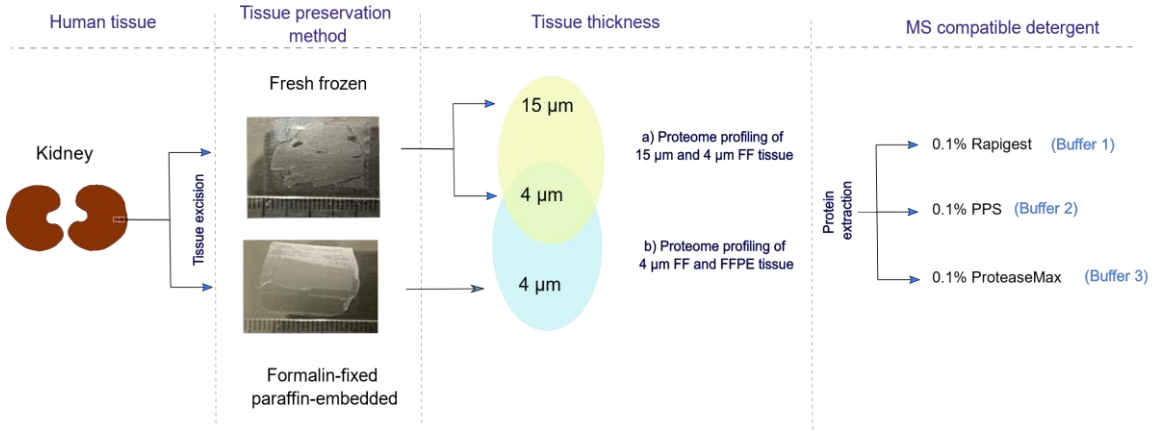

Figure 1. Scheme of the study of protein extraction from FF and FFPE tissues with MS-compatible buffers. Proteins were analyzed using 4 µm and 15 µm thick FF tissue to examine influence of the tissue thickness on the methods. Further, extracted proteins were compared between 4 µm thick FF and FFPE tissues to evaluate appropriateness of the minute amounts of the FFPE tissues to be used with the MS-compatible protocols (Taken from Dapic et al., 2022).

Description of results:

Detergents are often used as a component in extraction buffers and one of the most employed detergents used for this purpose is SDS, however, it has been shown that it can cause ion suppresion during MS measurements. Therefore in this study three different buffers that contained MS-compatible detergents: Rapigest, PPS Silent and ProteaseMax were used for protein extraction. Comparison of detected proteins from 15 μm thick FF and FFPE microdissected human kidney tissues showed that buffers containing Rapigest (630±80) and ProteaseMax (498±51) yielded the highest number of proteins identified for 15 μm tissue (1 mm^2). Used methods showed good reproducibility with correlation of the normalized spectral abundance factor (NSAF) values of the identified proteins between the biological replicates ranging from 0.76 to 0.89 for Pearson coefficients.

As expected fewer proteins were identified in corresponding samples of 4 μm tissue (2.5 mm^2), whereas most proteins were identified with buffer containing PPS Silent (265±44). Results showed similar sequence coverage for 4 μm and 15 μm tissue suggesting that buffers have similar efficiencies in solubilizing proteins for both tissue thicknesses. The physicochemical properties of the identified proteins extracted with Buffer 2 (containing PPS) were evaluated in terms of molecular weight (M_W) distribution, pI distribution, grand average of hydropathy (GRAVY) scores, cellular localization, and K/R ratio. Results showed that for both FF and FFPE tissues the most of the proteins were between 10 and 20 kDa mass range, and in terms of GRAVY scores the most abundant proteins were detected in the range −0.4 to −0.2. Cellular localization of the analyzed proteins showed that proteins in cytosol and nucleus had the highest abundancies for both tissue types. Somewhat higher abundancies of membrane proteins in FFPE tissues were observed, which might be indication of good solubilization properties of buffer containing PPS Silent. To further investigate chemical modifications the ratio of C-terminal lysine-containing peptides versus C-terminal arginine-containing peptides (K/R) were determined. These results showed that K/R was decreased for FFPE compared to FF tissues indicating underrepresentation of K residues probably due to the reaction of the side chains with formaldehyde that was also observed in other studies.

Further, it is known that urea is often added to the extraction buffers because it facilitates protein unfolding and representation to trypsin to improve protein digestion. However, while extraction of proteins in FFPE tissues needs to be carried out at elevated temperature (at 95°C to 100°C) to induce de-crosslinking of the proteins, high temperatures might promote protein carbamylation induced by urea. Here results on microdissected human kidney tissues showed that increasing incubation temperature promoted formation of carbamylated peptides which as a consequence had lower number of identified total proteins. Therefore, it is reccomended that tissues are first incubated in the extraction buffer at elevated temperatures (i.e. at 95°C) and after decreasing temperature of the buffer to 37°C to supplement buffer with urea.

The importance of results:

This study reported the influence of different chemical extraction procedures and physical parameters such as temperature on identification of proteins in biopsy size FFPE tissues. Results were compared to FF tissues and it was shown that isolated proteins had similar physicochemical properties. Moreover, it was shown that it is important to experimentally determine the best choice of the extraction buffer as this parameter might be tissue size and type dependent.

2. Pirog, A.; Faktor, J.; Urban-Wojciuk, Z.; Kote, S.; Chruściel, E.; Arcimowicz, Ł.; Marek-Trzonkowska, N.; Vojtesek, B.; Hupp, T. R.; Al Shboul, S.; Brennan, PM; Smoleński, RT; Goodlett, DR; **Dapic, I.*** Comparison of Different Digestion Methods for Proteomic Analysis of Isolated Cells and FFPE Tissue Samples. Talanta 2021, 233, 122568.
IF= 6.057; $IF_{5\text{-}year}$= 5.386; MNiSW= 100 pkt; citations (WoS: 6/4, Google scholar: 6)

Aim of the study:

Sample preparation is one of the key steps in proteome analysis, and methods used for sample analysis should be reliable, robust and have potential for implementation in clinical settings or automation. In this study several sample preparation protocols including filter-aided sample preparation (FASP), traditional in-solution digestion (ISD) and pressure assisted method with a Barocycler were tested for protein processing. Methods were tested on two different sets of biological samples which included isolated immune cell subsets (CD4+ T cells) and glioblastoma (GBM) FFPE tissue samples. Moreover, samples were independently analyzed in two different laboratories on two LC-MS platforms.

Description of results:

Results showed that the number and amount of identified peptides in both sample types, CD4+ T cells and FFPE tissues were lower in the samples processed with pressure cycling technology (PCT) as compared to ISD method. FASP method generated lower number and amount of peptides, however this did not lead to lower number of identified proteins, suggesting its effectiveness in digestion of the wide range of the proteins which might be resistant to digestion by other methods. To evaluate reproducibility of the methods the distributions of relative standard deviations (RSD) of measured abundances of quantified proteins were compared. Results showed that ISD and PCT method (93% and 96% of protein groups with RSD < 0.2, respectively) had somewhat higher reproducibility compared to PCT/ACN and FASP methods (87% and 91%, respectively). Further, results showed that specificity of digestion was highest for PCT/ACN and ISD in CD4+ cell samples while in the analysis of FFPE GBM tissues ISD method showed the highest digestion specificity (2.6% semitryptic peptides) followed by PCT and FASP (6.8% and 10.8% semitryptic peptides, respectively). Further, it was shown that number of misscleavages might be method dependent, whereas FASP showed the least misscleavages (6% in CD4+ cells and 29% in FFPE tissue) than ISD (22% in CD4+ cells and 45% in FFPE tissue) and PCT (33% in CD4+ cells and 47% in FFPE tissue). Results suggest higher trypsin activity in semi-solid phase used in FASP, and results also have shown that a lower amount of miscleaved peptides is not an effect of retaining longer peptides on a FASP membrane.

Moreover, FFPE related chemical modifications were investigated and results demonstrated significant increase of +14 Da modification which could be attributed to lysine side chains across all sample preparation workflows reflecting methylation introduced by a reaction of primary amino group with formaldehyde. Results also showed decreased K/R at C-terms of peptides and increased miscleavage rate. Trypsin digestion only rarely occured on modified lysine residues and almost no modified lysines were identified at the C-terminal position.

The importance of the results:

In this study several methods and independently analyzed two different types of samples (CD4+ cells and FFPE tissues) were investigated using two different LC-MS platforms. ISD method requires longer sample analysis time mostly related to long (overnight) digestion, however, it can be used as low-cost alternative to PCT and FASP methods. Moreover, personnel can be easily trained for ISD method and

it is easy to implement in laboratories. FASP methods showed a clearly different pattern of protein digestion and also showed the highest number of integral membrane proteins (IMP) detected. PCT method as assisted with high pressure in digestion of samples has the advantage of short sample preparation time and can lyse resistant samples. Moreover, extensive cross-linking introduced by preservation procedure in FFPE tissues has been shown to have an effect on modification of lysine residues (+14 Da). Therefore, it is reccommended to set this modification in the search engine to account for this modification.

3. Weke, K.; Singh, A.; Uwugiaren, N.; Alfaro, J. A.; Wang, T.; Hupp, T. R.; O'Neill, J. R.; Vojtesek, B.; Goodlett, D. R.*; Williams, S. M.; Zhou, M; Kelly, RT; Zhu, Y; **Dapic, I.*** MicroPOTS Analysis of Barrett's Esophageal Cell Line Models Identifies Proteomic Changes after Physiologic and Radiation Stress. Journal of Proteome Research 2021, 20, 2195-2205
IF= 4.466; $IF_{5\text{-year}}$= 4.352; MNiSW= 100 pkt; citations (WoS: 1/0, Google scholar: 1)

Aim of the study:

Microscale proteomics is an important tool for the analysis of proteins from samples of limited amount that are often present in clinic specimens. Here Microdroplet Processing in One pot for Trace Samples (microPOTS) technology was used for the analysis of the proteins from small number of the Barrett's oesophageal cells. With the microPOTS technology more than 1500 proteins were successfully identified from ~200 cells after exposure to chemical and radiation stress.

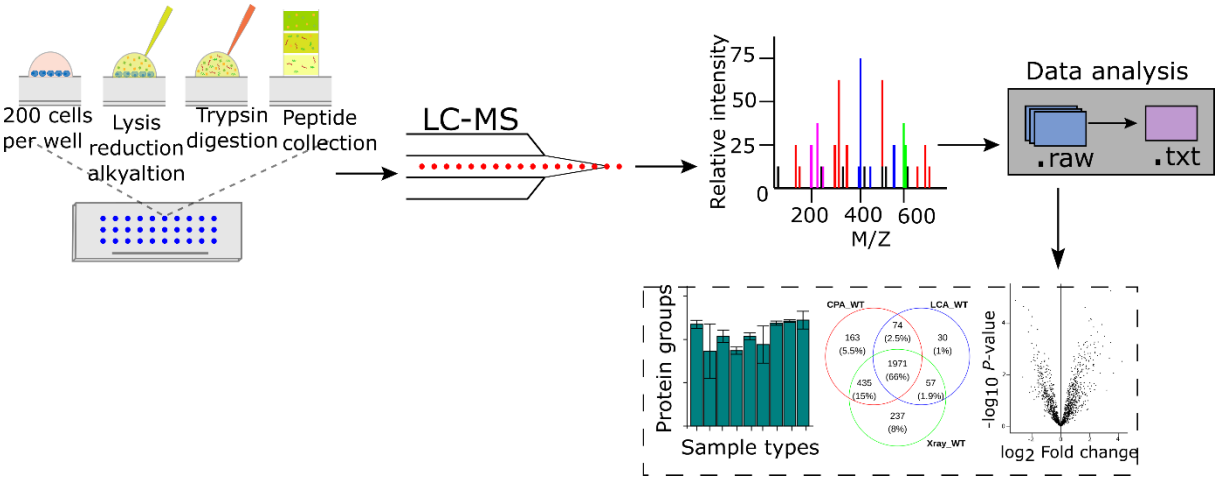

Figure 2. Cells were grown in keratinocyte media, and the generated CP-A null cells and wild-type were subjected to different stressors as previously described. Samples were then processed for protein extraction and further digested into peptides using the microPOTS system. The collected peptides were subsequently subjected to LC-MS/MS analysis. A spectrum showing the relative intensity and mass to charge ratio (*m/z*) of the ions being analyzed. The resulting files were loaded into MaxQuant for peptide identification, after which the output files of this step were next imported into R and analysed using the DEP package. A subset of the results after performing the analysis (Taken from Weke et al., 2020).

Description of results:

In this study microPOTS technology was used for the analysis of the proteins after their exposure to chemical and radiation stress. Experiments were performed on CPA wildtype (CP-A WT), p53 null (CP-A KO), and p53 Smad4 double null (CP-A dKO) cell lines which were further treated with 10 µM lithocholic acid (LCA) or irradiated with 2 Gy X-ray and cultured for further 24 hours. Results showed

more than 1500 proteins identified from approximatelly 200 cells from each sample group. More specifically average number of the identified proteins was as given in parenthesis: CPA WT (2273), CP-A KO (1673), CP-A dKO (2008), CP-A LCA-WT (1685), CP-A LCA-KO (2004), CP-A LCA-dKO (1821), CP-A X-ray-WT (2300), CP-A X-ray-dKO (2345), and CP-A X-ray-KO (2367). Total number of the proteins from three replicates of CP-A LCA-WT, CP-A LCA-KO and CP-A LCA-dKO increased to over 2000 for all investigated groups and produced high proitein overlap of 67% (1867 proteins) between the groups. Good tryptic digestion was indicated with over 75% of fully tryptic cleavage sites with fewer than 25% missed cleavages. Quantitative reproducibility between the samples was evaluated with LFQ values and demonstrated with high Pearson's correlation coefficient value ($R > 0.9$). Further investigation of physico-chemical properties of the detected proteins showed that high number of hydrophobic proteins were extracted with microPOTS technology.

Analysis of the differential expression of proteins between samples before and after LCA and radiation treatment revealed that stress induced changes in the proteins of CP-A cells. Detected proteins are known to have important biological roles and some proteins such as anterior Gradient 2 (AGR2) have a significant regulatory role and their expression has also been related to human cancers such as neoplasia of oesophagus. Further, ACBP was found to be increased in LCA treated cells (LCAdKO) that might be related to the role of bile salts in inducing oesophageal cancer and also is known for driving tumorigenesis in glioblastoma and lung cancer.

The importance of the results:

MicroPOTS method was shown to be applicable and sensitive enough to detect changes in the proteome signature after cells have been exposed to stress. This is of particular importance for the application of technology to patient's samples and to determine differential expression of important biomarkers. It could also be of particular use for analysis of proteomic signatures of so-called „grey zone", which represents border between tumorous and healthy tissue and it is often present in limited areas. Application of microPOTS based sample treatment to such samples might also reveal new potential target molecules to treat pathogenesis of cancer.

4. **Dapic, I.***; Baljeu-Neuman, L.; Uwugiaren, N.; Kers, J.; Goodlett, D. R.; Corthals, G. L.* Proteome Analysis of Tissues by Mass Spectrometry. Mass Spectrometry Reviews 2019, 38, 1–39.
IF= 8.887; $IF_{5\text{-}year}$= 8.479; MNiSW= 140 pkt; citations (WoS: 16/14, Google scholar: 22)

Aim of the study:

Review paper describes and gives detailed information about the existing methodologies for sample preparation of the biological material in bottom-up proteomics and subsequent LC-MS analysis. There have been numerous protocols reported for the analysis of tissues and yet there is no consensus on best practice procedures, which makes it difficult to compare results between different studies. Here I aimed to summarize protocols used in clinical proteomics of FF and FFPE tissues, and to compare them in terms of tissue types, tissue size and procedures used for their analysis.

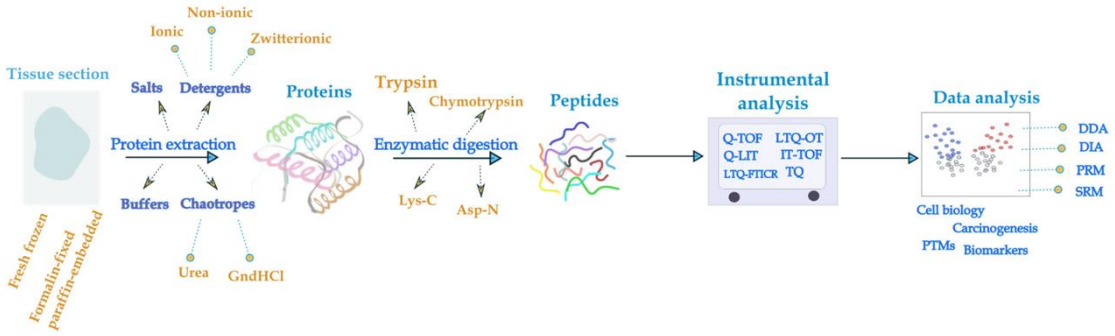

Figure 3. Typical tissue analysis workflow. Microdissected tissue sections can be obtained as FF or FFPE tissues. Tissue processing includes protein extraction followed by digestion to peptides enzymatically most often with trypsin. Next various cleanup methods are available for preparation for LC-MS/MS analysis. Bioinformatic analysis of the tandem MS data leads to identification of peptides and their corresponding proteins. Further, this information is used to answer biological question and to help in better understanding of biochemical processes behind the scene. DDA, data dependent acquisition; DIA, data independent acquisition; FF, fresh frozen; FFPE, formalin fixed paraffin embedded; IT-TOF, ion trap-time of flight mass spectrometry; LC-MS/MS, liquid chromatography tandem mass spectrometry; LTQ-FTICR, linear quadrupole ion trap Fourier transform ion cyclotron resonance mass spectrometry; LTQ-OT, linear trap quadrupole orbitrap mass spectrometry; PRM, parallel reaction monitoring; PTM, posttranslational modification; Q-LIT, linear quadrupole ion trap; Q-TOF, quadrupole-time of flight mass spectrometry; SRM, single reaction monitoring; TQ, triple quadrupole mass spectrometry (Taken from Dapic et al., 2019).

Description of results:

Patient tissues are preserved either as formalin-fixed parafin-embedded (FFPE) tissues, or as fresh frozen (FF) tissues. FFPE presents stadard method for tissue preservation, however during the fixation protocol with formaldehyde chemical modifications as lysine methylation (+14 Da) as a major modification, but also methylene (+12 Da) and methylol (+30 Da) modifications have been reported.[11,29]

In the review current methods used to improve recovery of proteins are discussed in respect to the tissue type and size. Successful method for protein extraction from FFPE tissues should remove formaldehyde-induced cross-links (as intra-chain and inter-chain methylene bridges), but also avoid formation of new chemical changes in proteins. This is mostly done at elevated temperature using the antigen retriveal procedure first described by Shi et al.[30] Buffers mostly used for the extraction of proteins from FFPE tissues are Tris-HCl, ammonium bicarbonate or RIPA buffer. The most frequently employed organic solvent used for protein extraction is acetonitrile in the range of concentration 20-80%[31–33] , however, some protocols also describe that the use of 2,2,2-trifluoroethanol (TFE) in the range of 40–50% improved solubilization and separation of membrane proteins detected with isoelectric focusing[34]. Surfactants or detergents are often used in protein extraction buffers, and they can be classified as ionic, non-ionic or zwitter-ionic. By far the most widely used is the ionic surfactant SDS, however, it is known as an ion supressor that reduces peptide detection and interferes with electrospray ionization. Importantly, alternatives that are known as MS-compatible detergents avoid ion supression with the most commonly being the cationic acid-labile surfactant Rapigest and ProteaseMax, and an anionic acid-labile surfactant PPS Silent. Urea is the most commonly used chaotrope for protein extraction, however, heating samples in the presence of urea can lead to carbamylation of amino and sulfhydryl groups, and

to chemical modification of lysine and arginine residues.[18,35,36] Protein modifications as a consequence might have incomplete digestion, shift in the retention time and poor chromatographic performace.
Moreover, there have been desribed technological developments that include physical and mechanical procedures for retriveal of proteins from biological materials. Mostly used are ultrasonication and pressure based systems or picosecond infrared laser (PIRL) that use thermal or mechanical energy with the goal to shorten long chemical extraction procedures or digestion times. The choice of techniques ultimately depends on the study goal. For example, PCT systems such as the Barocycler use a combination of heat and high pressure and have been succesfull in extraction of proteins from small tissue amounts. Finally, laser techniques have been widely used in surgery, and as one of them PIRL uses tissue ablation and minimizes thermal damage of the surrounding tissue, which was proven for protein extraction from muscle tissues. [37].

The importance of results:

Proteomic analysis of the microdissected tissues after histopathological inspection has made a significant contribution to mapping the tumour microenvironment. Intention of the review was to summarize procedures for mapping proteins in small tissue biopsies with regard to tissue preservation methods and chemical or physical procedures used for protein extraction. Thus, this review can be used as a guide for scientists who would like to inform themselves on current existing procedures, as well as on critical evaluation of the suitability of a particular method. Moreover, in the review it has been described in detail the effect of a buffer's components on the efficiency of protein extraction.

5. **Dapic, I.**; Uwugiaren, N.; Jansen, P. J.; Corthals, G. L. Fast and Simple Protocols for Mass Spectrometry-Based Proteomics of Small Fresh Frozen Uterine Tissue Sections. Analytical Chemistry 2017, 10769–10775.
   IF= 6.042; $IF_{5\text{-}year}$= 6.035; MNiSW= 140 pkt; citations (WoS: 14/10, Google scholar: 19)

Aim of the study:

In this study I developed analytical methods for fast and reliable analysis of FF human uterine tissue. Medical Centre in Leiden provided microdissected human uterine tissues at 10, 16 and 20 μm thickness and tissues were prepared for proteomic analysis using in-solution digestion with different buffers and digestion times.

Description of results:

Results showed that MS-related parameters of time of flight-mass spectrometry (TOF-MS) and TOF-MS/MS accumulation times had a significant effect on the number of identified peptides and proteins. The optimal instrumental TOF-MS and TOF-MS/MS accumulation times were found to be 500 and 100 ms respectively, and these parameters were used throughout the study. Extraction of proteins from 10, 16, and 20 μm thick FF human uterine tissue was tested with different protocols (Protocols 1 to 5, Figure 4). Results showed that Protocol 1 (containing 0.1% SDS/30% ACN/urea) resulted in fewer identified proteins compared to the other protocols. With Protocol 2 (containing 30% ACN/urea) 55% more proteins were identified in 10 μm thick tissue than Protocol 1, while the number of identified proteins was 20% higher when using Protocol 2 in 16 μm tissue. The lower number of identified proteins with the buffer containing SDS (Protocol 1) could be attributed to precipitation of proteins or incomplete removal of detergent. Further, reproducibility of the analysis was examined with NSAF values. Results showed good reproducibility of the protocols with Pearson $R \geq 0.78$ for 10 μm tissue.

To determine the minimum amount of tissue needed for quantitative analysis we analyzed 0.2, 0.6, 0.8, 1.2, 1.6, and 2 mm^2 of 10, 16, and 20 μm FF uterus tissue. An increase in the amount of analysed tissue led to an increase in the number of identified proteins for all examined tissue thicknesses. Results showed that an increase in tissue thickness led to an increase in the corresponding NSAF value and methods were sensitive enough to determine proteins from a small tissue amounts as 0.2 mm^2, and plateau in the number of the identified proteins was observed for the values between 1 mm^2 and 2 mm^2 of the tissue.

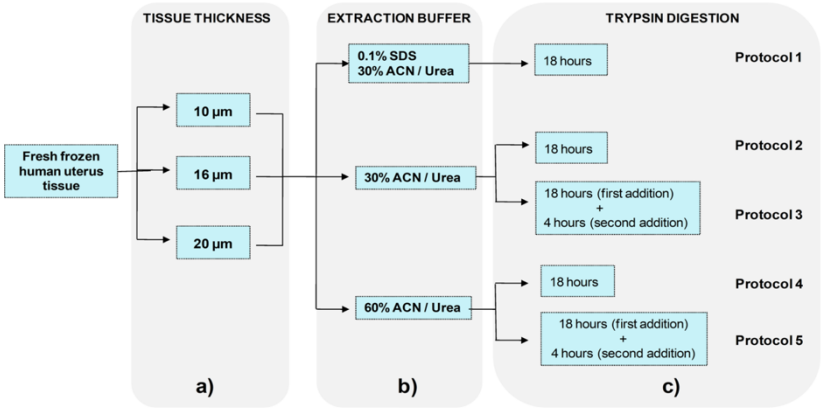

Figure 4. Schematic overview of the current study. (a) 10, 16, and 20 μm thick FF human uterus tissue were used in the study. (b) Proteins from the tissues were extracted using 3 different solutions varied by detergent (SDS) and organic solvent (ACN). (c) Protein digestion was performed over 18h using a single portion of trypsin (Protocols 1, 2, and 4) or for an additional 4 h after the addition of second portion of trypsin (Protocols 3 and 5) (Taken from Dapic et al., 2017).

The importance of results:

The analysis of small tissue biopsies is of great interest to clinicians with the respect to understanding a patient's samples and biopsies. Fewer proteins identified with the protocol containing SDS might lead to the conclusion that SDS solubilizes well a subset of the proteome, but not all the proteins as with the other methods that we have tested. This is of particular importance because SDS is usually used for the extraction of hydrophobic proteins due to their lower solubility in extraction solvents. Furthermore, tested methods showed good reproducibility and an additional digestion with trypsin showed higher overlap between the replicates.

6. Wouters, B. [†*]; **Dapic, I.** [†]; Valkenburg, T. S. E.; Wouters, S.; Niezen, L.; Eeltink, S.; Corthals, G. L.; Schoenmakers, P. J. A Cyclic-Olefin-Copolymer Microfluidic Immobilized-Enzyme Reactor for Rapid Digestion of Proteins from Dried Blood Spots. Journal of Chromatography A 2017, 36–42. IF= 3.716; $IF_{5\text{-year}}$= 3.713; MNiSW=100 pkt; citations (WoS: 17/16, Google scholar: 25)

Aim of the study:

Enzymatic digestion of proteins is one of the most critical steps in sample preparation for bottom-up proteomics. The typical way of performing in solution digestion is mixing solution of proteins with enzymes in a specific ratio; e.g. 1:100 enzyme:sample. Digestion is usually carried out overnight, which makes methods long and limits high throughput of the analysis.Therefore, the main aim of this study was to develop a device immobilized enzyme reactor (IMER), that could be used to shorten protein digestion time. IMER was successfully used on model proteins and dried-blood-spots (DBS) shortening digestion times from overnight to several minutes.

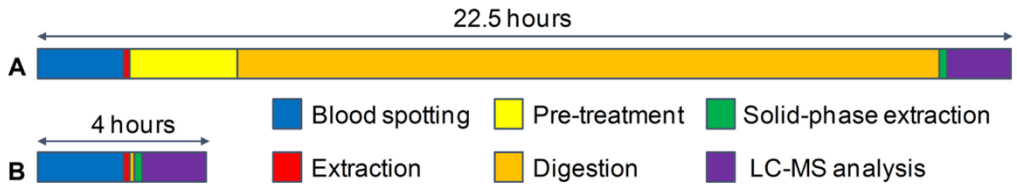

Figure 5. Comparison of duration of traditional workflow including in-solution digestion (A) with the novel IMER-facilitated workflow (B). IMER-facilitated workflow consists of 5 steps, omitting the pre-treatment step, and reduces the total analysis time to 4 h (Taken from Wouters et al., 2017).

Description of results:

To facilitate digestion of the protiens a cyclic-olefin-copolymer (COC) microfluidic reactor containing trypsin immobilized on a polymer monolithic material via azlactone chemistry was developed. This type of microfluidic reactor was chosen due to the low cost, good chemical resistance to a wide range of solvents and transparency in visible and ultraviolet light regions. The microfluidic device had two bonded layers of micro-machined cyclic-olefin copolymer (COC) and contained a microchannel with a length of 60 mm and 300 μm diameter circular cross-section. IMER was used for offline digestion of several proteins of different molecular weight as cytochrome c (11.7 kDa), myoglobin (17 kDa), S1-casein (23 kDa), ovalbumin (42.8 kDa), bovine serum albumin (66.5 kDa), transferrin (75.2 kDa), and catalase (240 kDa). Proteins were digested with no protein pre-treatment and digestion was carried out in 293 seconds. Results were compared for proteins digested with IMER and for proteins digested with traditional overnight digestion. IMER digestion showed slightly higher sequence coverage for proteins with lower molecular weight (cytochrome c, myoglobin, S1-casein, ovalbumin). A higher number of disulfide bridges in the proteins that had higher molecular weight showed higher sequence coverage when overnight digestion was performed as compared to IMER (bovine serum albumin showed 55% and 95% for IMER-digestion and in-solution digested, respectively) and transferrin (56% and 78% for IMER-digestion and in-solution digestion, respectively). Size of the proteins also plays important role in digestion, probably due to the effect of exposing cleavage sites to the enzyme. Results for catalase somewhat lower IMER digested sequence coverage (75%) compared to in-solution digestion (88%). In further study IMER was used for digestion of proteins extracted from DBS. IMER-facilitated digestion revealed 142 proteins from DBS that were digested in only 5.6 minutes. The identified proteins were compared to traditional overninght digestions of proteins with results showing a similar molecular weight distribution and GRAVY value for protein sequences.

The importance of results:

Development of the microfluidic reactor significantly shortened sample preparation from 22.5 hours for in-solution digestion to 4 hours when IMER-facilitated digestion was used. Similar physico-chemical properties of the proteins digested with IMER and in-solution digestion showed potential to use such devices in proteomics, thus allowing faster digestion of the proteins and higher throughput of the samples.

5. Presentation of other scientific and research achievements including teaching, organizational achievements and science popularization

5.1. Research and scientific achievements before doctoral degree

I finished my studies and graduated in physical chemistry in 2008 at the Department of Chemistry, Faculty of Science, University of Zagreb. My master's thesis research work was focused on investigation of the properties of the plyelectrolyte-proteins multilayers after their adsorption on silica tile or silica nanoparticles. Here I have investigated with optical reflectrometry how different conditions such as charge, ionic strength of the solution and concentration of the polyelectrolytes influence formation and stability of adsorbed multilayers. Results showed that adsorption of bovine serum albumine (BSA) depends not only on the concentration of the protein, but also on the anchoring level poly(allylamine hydrochloride)/poly(sodium 4-styrenesulphonate) (PAH/PSS) multilayer with PAH being a terminal layer.[38,39] During my last year of graduate studies I participated in the IAESTE students exchange programme during which I spent three months in Dynea AS Norway and Lillestrøm, Norway. Under supervision of Kristina Durkic, Senior development chemist in Dynea who trained me in R&D Laboratory analysis of resins. During undergraduate studies I was also involved in laboratory research in inorganic chemistry for which I received the **Rector's award** from the University of Zagreb (Title of the work: „Synthesis and identification of products of reactions of chromium (II) with salycilaldehyde 4-phenyl-3-thiosemicarbazones“, 2007).

After I have graduated I started a position of Scientific and Research assistant at the Faculty of Food Technology and Biotechnology, University of Zagreb. Also at the same time I started PhD studies in Analytical chemistry at the Faculty of Science, University of Zagreb. The primary goal of my PhD project was development and validation of tools for detection of biochemical indicators in impaired skin barrier, i.e. atopic dermatitis (AD) obtained after tape stripping. For this work I developed an LC-UV method for quantification of amino acids and their derivatives in the outermost layer of skin, so-called stratum corneum. In collaboration with Dr Sanja Kezic I spent time in Coronel Institute of Occupational Health, Academic Medical Center, Amsterdam, Netherlands where I was trained in high performance liquid chromatography and determination of natural moisturizing factors in human stratum corneum. This collaboration resulted in several joint papers that were focused on quantitation of so-called natural moisturizing factors (histidine, pyroglutamic acid, *trans*- and *cis*-urocanic acid and tyrosine) that play an important role in hydration and homeostasis of the skin (Dapic et al., 2013)[40]. Method was successfully applied to several patient studies indicating significantly different levels of NMFs in chemically damaged skin and controls (Angelova Fischer et al., 2014; 2015)[41,42] and after exposure to UV (Landeck et al., 2016)[43]. In the next steps of my doctoral thesis I assumed that damaged skin has altered composition of stratum corneum lipids, more specifically I have focused on free fatty acids present in the skin. Therefore, I developed an LC-MS method based on “surrogate analyte” quantification of eight long and very long chain FFAs (C12-C28) (Dapic et al., 2017).[44] Method was used on normal and AD skin revealing differences in FFAs amounts. The results showed an important role of FFAs in skin permeability and proper function. For this method I received **„The Ronnie Marks“ award** given by the International Society for stratum corneum research (2014). Also, in 2012 I received funds for collaborative two weeks visit and training at Faculté de Médecine et de Pharmacie, Lyon, France where under supervision of dr. Marek Haftek and dr. Jacques Portoukalian I learned isolation of ceramides and other lipid classes from human epidermis (2012). Further, I have also worked on determination of the structure of ceramides (CER) in the skin and in further work I have also used LC-

MS to elucidate structure of some ceramides using hydrogen-deuterium exchange mass spectrometry (Dapic et al., 2019).[45]

5.2. Research and scientific achievements after doctoral degree

After finishing PhD studies I moved to Amsterdam where I performed postdoctoral research at the van 't Hoff Institute for Molecular Sciences in Biomolecular Systems analytics group under supervision of prof Garry Corthals (2015-2017). During postdoctoral research there I closely collaborated with pathology departments for development of the tools to improve pathological observations. Here I have particularly focused my research on analysis of limited amounts of human material in collaboration with pathology departments. To do so I investigated different approaches for quantitation and detection of proteins, including cross-linking[46], isobaric tags for relative and absolute quantitation (iTRAQ), cysteine-targeted enrichment of proteins from human tissues and new technologies for selective isolation of phosphorylated peptides. I supervised development of computational tools for optimisation of data independent acquisition (DIA) and their application to SWATH-MS measurements of human uterine and kidney tissues (Florian Lucas, Computational tools to aid mass-resolved proteome measurements, MSc thesis, 2017; University of Amsterdam; supervisors: I. Dapic, B. Balluff and G. Corthals). Developed model used machine learning for prediction of flexible *m/z* windows and showed significant increase of peptide identification, and protein reproducibility compared to traditional SWATH-MS with fixed *m/z* windows.

Since July 2018 I have been employed as the Chemical Biology Group Leader at International Centre for Cancer Vaccine Science, University of Gdansk. Here my scientific work is focused on development of technologies for detection of potential biomarkers and neoantigens in different tumour types (glioblastoma, non-small cell lung carcinoma) as potential therapeutic targets. Notably during this time I received an instrumental grant for Orbitrap Exploris 480 mass spectrometer from Polish Science Foundation for the group to being to conduct experimental work. Here I investigated application of DIA-MS for screening and detection of potential biomarkers in formalin-fixed paraffin-embedded (FFPE) human glioblastoma (GBM) tissues (Weke et al., 2022)[47]. In this study DIA-MS workflow was successfully applied to patient-derived microdissected GBM tissue samples. Proteins were detected with the awarded Orbitrap Exploris 480 that combines high-resolution, accurate-mass, ultra-high-field Orbitrap analyzer with high performance quadrupole precursor ion selection. To this end the Chemical Biology Group uses MS as a tool for proteome profiling of human tissues (Pirog et al., Weke et al.)[47,48] and other biological materials, with the perspective to understand signalling within the tumoral microenvironment. Further, I coordinated publication of the book chapter „Trends in Sample Preparation for Proteome Analysis In Mass spectrometry“ demonstrating novelties and challenges of proteins detection (Editor: G. Mitulovic, IntechOpen, 2021, corresponding author Irena Dapic).

Development of microscale analysis is necessary to profile cellular subsets and to take advantage of the single pot sample processing microPOTS and nanoPOTS technologies were used in collaboration with group of Drs Kelly (Brigham Young University) and Zhu (Pacific Northwest National Laboratory, PNNL) who developed nanoPOTS. In collaboration with colleagues from PNNL application of nanoPOTS technology was demonstrated for top-down detection of the proteins where reproducibly and quantitatively several hundreds of proteoforms from ~70 to ~770 HeLa cells was determined. Also, a variety of post-translational modifications were identified with less than 800 cells used.[49]

Moreover, in the Chemical Biology Group a better understanding of neoantigen isolation methods and their role in immune-based therapies was investigated. The challenges associated with mass

spectrometry detection of neoantigens was described in review paper "Mass Spectrometry-Based Identification of MHC- Associated Peptides"[50], while I also collaborated in molecular dynamics study of structural determinants of peptide-dependent TAP1-TAP2 transit passage.[51]

5.3 Teaching achievements

Already as a third year student at the Department of Chemistry, Faculty of Science at University of Zagreb I participated in teaching as demonstrator in laboratory work at the course „Laboratory exercises in physical chemistry". Here I helped students to conduct their exercises, as well as to better understand the theory behind them. After employment at Department of Chemistry and Biochemistry at Faculty for Food technology and Biotechnology, University of Zagreb I taught first year students of Food technology and biotechnology and Nutricionism in laboratory work and seminars of the course „Introduction to chemistry and chemical analysis" (2009-2015) and teaching laboratory exercises and seminar classes (approx. 80 students, 2018).

During postdoctoral studies at van 't Hoff Institute for Molecular Sciences, University of Amsterdam (2015-2017) I was involved as lecturer in several teaching courses: Proteomics in OMICS, Advanced Separation Science Course, Analytical Chemistry in BSc. Also I was invited to give a lecture „Sample preparation for LC-MS proteomics studies" to the students at the ASTP Chromatography and Hyphenation Summer Course (24 AUG 2016). At the van 't Hoff Institute for Molecular Sciences I also supervised several BSc and MSc students during experimental work for their thesis, as well as several students who were performing their Literaure theisis or Project Scheikunde (2 MSc thesis, 5 BSc thesis, 3 Literature thesis, 3 Project Scheikunde, for a list of those projects please refer to Annex 5). Since employment at ICCVS I have been involved in supervision of projects of several PhD students and postdoctoral researchers.

5.4. Organizational achievements and science popularization

Since employment at the ICCVS as Chemical Biology Group Leader I was directly involved in setting up the infrastructure of the Centre, and organisation of laboratories. This particularly included administration that was required for setting up the laboratory (employment of PhD students and postdoctoral researchers, public tenders for laboratory equipment, organization of the laboratory space and instruments). Moreover, I organised a weekly Journal Club meeting at the International Centre for Cancer Vaccine Science where scientists from the ICCVS and collaborators would present their projects and discuss further ideas. For attendance of this meeting PhD students were also accredited ECTS points.

I also participated in the organization of several conferences in mass spectrometry: member in organization of Mass Spectrometry in Biotechnology and Medicine (MSBM X) summer school (3-9 July 2016, Dubrovnik, Croatia); member in the organization of the Core Facilities Workshop on Human proteome organization conference (HUPO, 17 September 2017, Dublin, Ireland); member of the Organising committee for OurCon V conference (Mass spectrometry imaging conference held 25-28 September 2017 in Doorn, the Netherlands), role: scientific programme responsible; I was also the main Scientific organiser of the workshop Jesienne Sympozjum Naukowe 2018 Interdisciplinarity in Cancer Neoantigen Science (Gdańsk, 14-15 November 2018).

I was a member of the Committee for informatics, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb (2011-2013). Currently, I am a member of Advisory board of Rapid communications in mass spectrometry (2018-).

In my career I have also been involved in science popularization activity where I had a chance to share my passion for science with the wider community. I held workshops at the Summer Science Factory (AUG 2011 Split, Croatia; July 2013 Samobor, Croatia) and I also participated at the Scientific picnic in SEP 2012, Zagreb, Croatia where through experiments and discussions science was brought closer to public.

Scientific achievements:

• Total number of publications: 21
Publications from research work after doctoral degree: 14
Publications from research for doctoral degree: 5
Publications from MSc: 2

(the first author of 9 papers; corresponding author of 7 papers); *publications in which i am first and corresponding author were counted towards first author publications.*

• **Scientific achievement: 6 publications** (the first author of 4 publications; corresponding author of 2 publications) IF = 33.580, MNiSW = 680 pkt**;** *publications in which I am the first and corresponding author were counted towards first author publications*.

• **The total number of publications excluding scientific achievement: 15** (the first author of 5 papers; corresponding author of 2 papers) IF= 54.142; $IF_{5\text{-year}}$ = 52.839; MNiSW= 1210 pkt; *publications in which I am the first and corresponding author were counted towards first author publications.*

-Publications from MSc research: 2 papers, IF =3.543; $IF_{5\text{-year}}$ = 3.793; MNiSW=120 pkt
-Publications from research for doctoral degree: 5 papers, IF = 14.781; $IF_{5\text{-year}}$ = 13.120; MNiSW=350 pkt
-Publications from research work after doctoral degree: 14 papers, IF = 69.398; $IF_{5\text{-year}}$ = 68.449; MNiSW = 1420 pkt
•**The total number of MNiSW points**: 1890 (works before doctoral degree and for doctoral degree: MNiSW= 470 pkt; works after doctoral degree: MNiSW = 1420 pkt).
•**Number of citations (Web of Science): 265, (Google Scholar): 376**
•**Hirsch Index: Web of Science: 11; Google Scholar: 11**
• The total number of conference reports: 23 (3 before doctoral degree; 9 from doctoral degree; 11 after doctoral degree);

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