Dr Paulina Małgorzata Czaplewska

Summary of the Professional Scientific Achievements

Laboratory of Mass Spectrometry
Intercollegiate Faculty of Biotechnology

University of Gdansk and Medical University of Gdansk

Gdańsk 2018

1. **Name:** Paulina Małgorzata Czaplewska
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2. **Contact details:**

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3. **Awarded Diplomas and Degrees:**

   2005 r. PhD in chemistry, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland

   2001 r MSc in chemistry, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland

4. **Title of the PhD thesis, names of Supervisors and Reviewers:**

   Title: Synthesis, conformational and aggregational studies of beta amyloid peptides.

4.1 Publications included in the PhD thesis:

The value of Impact Factor (IF) downloaded from the website Web of Science (WoS), if IF was not present for the specific year, the IF for previous year was used, Ministerial Publication Points according to the List of Publications prepared at the Ministry of Science and Higher Education for a specific year, citation number (Lc) according to the Web of Science Core Collection database

P. Juszczyk, AS. Kołodziejczyk, Z. Grzonka.(2005) Circular dichroism and aggregation studies of amyloid beta (11-28) fragment and its variants. **Acta Biochimica Polonica**. **(IF_{2004} = 1.032, $IF_{3\text{-letni}}$ = 0.754, Punkty MNiSW 15, Lc = 14)**

Individual contribution of this work I estimated at 50%: *I am the first author of this manuscript, I performed all the experiments and participated in data analysis. I prepared all figures and tables, participated in writing and all corrections of the following versions of this manuscript.*

Summarized Impact Factor (IF) of publications belonging to the PhD thesis = **1.032**

Summarized 5-year Impact Factor (IF 5-year) of publications belonging to the PhD thesis = **0.754**

Summarized value of the Ministerial Publication Points for publications belonging to the PhD thesis = **15**

4.2 Promotor and Reviewers of the PhD thesis

Promotor: prof. Zbigniew Grzonka University of Gdańsk

Reviewers: prof. Krzysztof Rekowski University of Gdańsk

prof. Zbigniew Szewczuk University of Wrocław

5 Information on so-far employments in Research Institutions:

2016 - present	**Assistant Professor** (adiunkt), Laboratory of Mass Spectrometry, Core Facillity, Intercollegiate Faculty of Biotechnology, University of Gdansk – Medical University of Gdansk
2013-2016	**Postdoc:** internship, mass spectrometry specialist FP 7 REGPOT *"MOBI4Health-Centre of Molecular Biotechnology for healthy life and environment"* Laboratory of Mass Spectrometry, Core Facility, Intercollegiate Faculty of Biotechnology, University of Gdansk – Medical University of Gdansk
2007-2013	**Assistant Professor** (adiunkt), Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdansk
2006-2007	**Postdoc:** internship, Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, Department of Chemistry, University of Konstanz
2005-2006	**Postdoc:** internship at the Department of Molecular Medicine University of Texas Health Science Center at San Antonio USA

6 Scientific Achievement as defined by Article 16, Clause 2 of the Act of March 14, 2003 on Academic Degrees and Titles as well as Degrees and Titles in the Arts (Journal of Laws No. 65, item 595, with subsequent amendments)

c) Title of Scientific Achievement:

Mapping of human cystatin C interactions with biologically active ligands.

d) Research publications belonging to the Scientific Achievement:

The Scientific Achievement (a monothematic cycle) includes six scientific publications (1 review publication and 5 experimental publications) collected under the common title as shown in Section 1 a) **(* - indicates corresponding author)**

The value of Impact Factor (IF) downloaded from the website Web of Science (WoS), if IF was not present for the specific year, the IF for previous year was used, Ministerial Publication Points according to the List of Publications prepared at the Ministry of Science and Higher Education for specific year, citation number (Lc) according to the Web of Science Core Collection database

1. M. Rafalik, M. Spodzieja, A.S. Kołodziejczyk, S. Rodziewicz-Motowidło, A. Szymańska ,A. Grubb, P. **Czaplewska*** (2018) The identification of discontinuous epitope in the human cystatin C – monoclonal antibody hCC3 complex. ***Journal of Proteomics,*** (**DOI**: **doi.org/10.1016/j.jprot.2018.04.020**)

   IF_{2017} = 3.914, $IF_{5\text{-year}}$ = 3.926, MNiSW = 35, L_c = 0

   Individual contribution to this work I estimate at 40*%: I am a correspondent author of the manuscript, I had a leading participation in the planning of all experiments and analysis of all results, I carried out the registration of all MALDI TOF spectra for the analyzed samples, I wrote a manuscript, prepared figures and tables, and prepared work for publication. In addition, I provided funding for research from my own project.*

2. M. Spodzieja, K. Kalejta, AS. Kołodziejczyk, M. Maszota-Zieleniak, S. Rodziewicz-Motowidło, W. Żmudzińska, **P. Czaplewska***. **(2016)** Characteristics of C-terminal, *β*-amyloid peptide binding fragment of neuroprotective protease inhibitor, cystatin C. ***Journal of Molecular Recognition***. 30(2): 1-13 **(DOI: 10.1002/jmr.2581)**

   IF_{2015} = 2.091, $IF_{5\text{-year}}$ = , 2.051, MNiSW 20, L_c = 2

   Individual contribution to the creation of this work I estimate at 35%: *I am the correspondent author of the manuscript, I had a leading part in the planning of all experiments and analysis of all obtained results, I carried out the registration of all mass spectra for the analyzed samples, wrote a manuscript, prepared figures and tables and prepared work for publication. In addition, I provided funding for research from my own project.*

3. M. Prądzińska, I. Behrendt, J. Astorga-Wells, A. Manoilov, R. Zubarev, A.S. Kołodziejczyk, S. Rodziewicz-Motowidło, **P. Czaplewska* (2016)**. Application of amide hydrogen/deuterium exchange mass spectrometry for epitope mapping in human cystatin C. ***Amino Acids*** 48(12): 2809-2820 **(DOI: 10.1007/s00726-016-2316-y),**

   IF_{2015} 3.29, $IF_{5\text{-year}}$ = 3.241, MNiSW 30, L_c = 6

   Individual contribution to the creation of this work I estimate at 40%: *I am the correspondent author of the manuscript, I had a leading part in the analysis of all obtained results, wrote a manuscript, prepared figures and tables and prepared work for publication. In addition, I provided funding for research from my own project.*

4. M. Prądzińska, I. Behrendt, M. Spodzieja, AS. Kołodziejczyk, S. Rodziewicz-Motowidło, A. Szymańska, SL. Lundström, RA. Zubarev, K. Macur, **P. Czaplewska* (2016)**. *Isolation and characterization of autoantibodies against human cystatin C.* ***Amino Acids*** 48(11): 2501-2518 (**DOI 10.1007/s00726-016-2271-7**)

   IF_{2015} 3.29, $IF_{5\text{-year}}$ = 3.241, MNiSW 30, Lc = 1

   Individual contribution to the creation of this work I estimate at 40%: *I was a correspondent author of the manuscript, I had a leading contribution in the planning of all experiments and analysis of all obtained results, I carried out the*

registration of mass spectra for the analyzed samples, I wrote a manuscript, prepared figures and tables and prepared a manuscript for publication. In addition, I provided funding for research from my own project.

5. I. Behrendt, M. Prądzińska, M. Spodzieja, AS. Kołodziejczyk, S. Rodziewicz-Motowidło, A. Szymańska, **P. Czaplewska* (2016)**. *Epitope location for two monoclonal antibodies against human cystatin C, representing opposite aggregation inhibitory properties.* ***Amino Acids*** 48(7): 1717-1729 (**DOI: 10.1007/s00726-016-2242-z**)

IF_{2015} 3.29, $IF_{5\text{-year}}$ = 3.241, MNiSW 30, Lc = 2

Individual contribution to the creation of this work I estimate at 40%: *I was a correspondent author of the manuscript, I had a leading contribution in the planning of all experiments and analysis of all obtained results, I carried out the registration of all mass spectra for the analyzed samples, I wrote a manuscript, prepared figures and tables and prepared a manuscript for publication. In addition, I provided funding for research from my own project.*

6. M. Maszota, N. Karska, M. Spodzieja, J. Ciarkowski, AS. Kolodziejczyk, S. Rodziewicz-Motowidlo, P. **Czaplewska***. *(2015) Structural studies of the C-terminal 19-peptide of serum amyloid A and its Pro -> Ala variants interacting with human cystatin C.* ***Journal of Molecular Recognition*** 28(7), 413-426 **(DOI: 10.1002/jmr.2457)**

IF_{2014} 2.091, $IF_{5\text{-year}}$ = 2.051, MNiSW 20, Lc = 1

I estimate an individual contribution to the creation of this work at 30%: *I am a correspondent author of the manuscript, I had a leading part in planning of all experiments and in analysis of all received results, I wrote a manuscript and prepared a manuscript for publication. In addition, I provided funding for research from my own project.*

7. M. Spodzieja, M. Rafalik, A. Szymańska, AS. Kołodziejczyk**, P. Czaplewska***. (2013) *Interaction of serum amyloid A with human cystatin C – asessment of amino acid residues crucial for hCC-SAA formation (part II).* ***Journal of Molecular Recognition.*** 26(9): 415-425 **(DOI: 10.1002/jmr.2283)**

IF_{2012}=3.31, $IF_{5\text{-year}}$ = 2.051, MNiSW 20, Lc = 8

Individual contribution to the creation of this work I estimate at 40%: *I was a correspondent author of the manuscript, I had a leading part in the planning of all experiments and in analysis of all obtained results, I carried out the registration of all mass spectra for the analyzed samples, I wrote a manuscript, prepared and developed figures and tables and prepared a manuscript for publication. In addition, I provided funding for research from my own project.*

8. M. Spodzieja, A. Szymańska, A. Kołodziejczyk, M. Prądzińska, M. Maszota, P. Stefanowicz, Z. Szewczuk, A. Grubb, **P. Czaplewska***. (2012) *Interaction of serum amyloid A with human cystatin C—identification of binding sites***.** ***Journal of Molecular Recognition*** 25(10):513-524 **(DOI: 10.1002/jmr.2220)**
IF_{2011}=3.31, $IF_{5\text{-year}}$ = 2.051, MNiSW 20, Lc = 9

Individual contribution to the creation of this work I estimate at 40%: *I was a correspondent author of the manuscript, I had a leading part in the planning of all experiments and in analysis of all obtained results, I carried out the registration of all mass spectra for the analyzed samples, I wrote a manuscript, prepared figures and tables and prepared a manuscript for publication. In addition, I provided funding for research from my own project.*

9. A. Śladewska, A. Szymańska, M. Kordalska, A. Lewandowska, A S. Kołodziejczyk, A. Grubb, G. Paraschiv, M. Przybylski, **P. Czaplewska***. (2011) *Identification of the epitope for anti-cystatin C antibodies (Cyst-13).* ***Journal of Molecular Recognition*** 24(4):687-99 **(DOI: 10.1002/jmr.1100)**
IF_{2011}=2.286, $IF_{5\text{-year}}$ = 2.051, MNiSW 20, Lc = 13

Individual contribution to this work I estimate at 40%: *I am a correspondent author of the manuscript, I had a leading role in planning of all experiments and analysis of all results, I made all measurements of mass spectra, wrote a manuscript, prepared figures and tables and prepared a manuscript for publication. In addition, I provided funding for research from my own project.*

10. **P. Juszczyk**, G. Paraschiv, A. Szymańska, A.S. Kolodziejczyk, S. Rodziewicz-Motowidlo, Z. Grzonka, **M. Przybylski***. (2009) *Binding epitopes and interaction structure of the neuroprotective protease inhibitor cystatin C with beta-amyloid revealed by proteolytic excision mass spectrometry and molecular docking simulation.* **Journal of Medicinal Chemistry** 52(8): 2420-2428 **(DOI 10.1021/jm801115e)**

IF_{2008} = 4.898, $IF_{5\text{-year}}$ = 5.896, MNiSW 45, Lc = 28

Individual contribution to the creation of this work I estimate at 40%: *I am the first author of the manuscript, I had a leading role in planning of all experiments and analysis of all obtained results, I made the following laboratory experiments: determination of binding sites for cystatin C and beta amyloid peptide using epitope excision and extraction mass spectrometry approach, affinity tests using microcolumns and ELISA methodology, I synthesized some of the peptides presented in the work, wrote a manuscript, prepared and figures and tables.*

Summarized Impact Factor (IF) of publications belonging to the Scientific Achievement = **31.770**

Summarized 5-year Impact Factor (IF5-year) of publications belonging to the Scientific Achievement = **29.800**

Summarized value of the Ministerial Publication Points for publications belonging to the Scientific Achievement = **270**

e) Description of the research aims and results of the above-mentioned Scientific Achievement along with a description of their potential applications:

8. Introduction

Protein-protein interactions as well as peptide-protein interactions are the basis of almost all physiological processes in the human body. They are also responsible for many pathological processes, like for example, neurodegenerative diseases which are a growing problem in highly developed and aging societies. Scientific research conducted in this field is focused on understanding the molecular mechanisms of protein complex formation and description of all factors affecting stability of the protein-protein complexes. This also opens the way to use ligands or their peptide fragments to control the physiological and pathological processes taking place in our body. **The purpose of my research was to identify fragments of human cystatin C, which are important for their interactions with some biologically active peptides and proteins.**

Human cystatin C (hCC, Figure 1 A) is a small protein (120 Aa) which attracts constant interest of many scientists. It happens mainly because of the main role of the mentioned protein in regulation of the activity of cysteine proteases[1]. Because of its almost universal presence in body fluids, hCC can affect both intracellular processes, such as apoptosis or antigen presentation, and extracellular ones, which include atherosclerosis, tumor metastasis and invasion of pathogens[2]. Interestingly, there is growing data on the involvement of cystatin C in processes independent of its inhibitory properties. In this type of activity, the modulator role of hCC is closely related to its interactions with other proteins; it can relate to:

- modulation of the activity of the transforming growth factor beta receptor, TGF-β[3]; blocking of signaling pathways in healthy and cancerous cells[4];
- influence on overproduction of proteins, e.g., promoter of GFAP acidic fiber protein in cultures of nerve cells[5];
- participation in the process of amyloidogenesis (hCC, and in particular its naturally occurring variant L68Q, as the main pathological factor in hereditary cerebral amyloid angiopathy HCCAA)[6];
- neuroprotective function associated with the inhibition of beta amyloid aggregation (Aβ)[7];
- protection of nerve cells against amyloid toxicity and increased survival of nerve cells in cultures in the presence of oligomeric or fibrillar forms of Aβ[8].

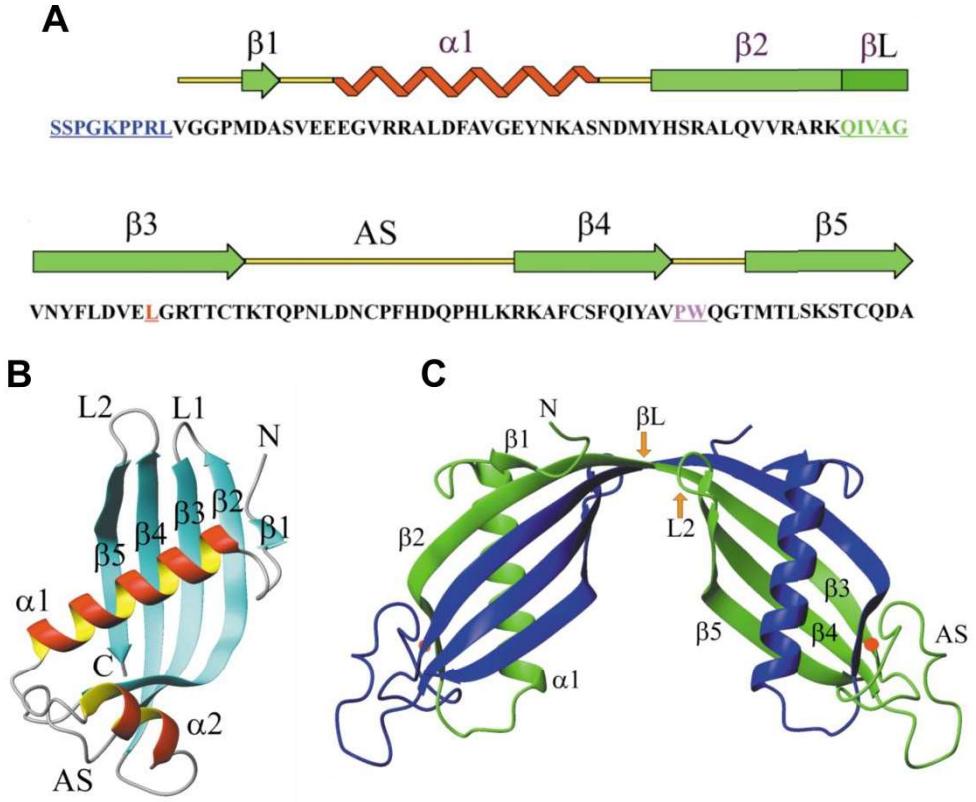

Figure 3 *The primary, secondary and tertiary structure of human cystatin C; A. amino acids sequence with marked secondary structure elements, in blue - fragment not present in crystallographic structure, in red α-helix, in green β strands; B. chicken cystatin C (PDB 1CEW), L1 loop 1, L2 loop 2; AS fragment called Appendix Structure; C. dimer of cystatin C*[9].

Due to its intra- and extracellular presence, hCC has the ability to interact with many proteins, which further can affect signaling pathways, and by interacting with elements of cell structures - their biological functions. Cystatin C is also an excellent starting point for the design of new biologically active compounds that are based on hCC binding sites with other proteins. In this way, up to now, it was possible to design and synthesize new inhibitors of cysteine proteases as well as peptidomimetics with a strong antibacterial and antiviral activity[10,11].

Human cystatin C and amyloid beta peptide (Aβ)

In contrast to the endemic nature of amyloidosis associated with cystatin C, aggregation of the amyloid β peptide (Aβ), which is the cause of Alzheimer's disease (AD), is more often recognized as a civilization disease. Every year, the number of older people suffering from progressive memory problems related to sporadic or hereditary form of the disease increases, and significantly changes the quality of the elderly people's life.

The main component of the amyloid deposits emerging in the neurodegenerative process is the Aβ peptide which consists of 39 to 42 amino acid residues that arises due to the improper processing of the amyloid

precursor protein (APP)[12]. As a result of the action of two enzymes, β and γ-secretases, the aggregation-prone peptides are released from APP, of which the 42-amino acid long one presents the highest tendency for fibrils formation. Although many research groups investigate the Aβ aggregation mechanism, there is no agreement on the mechanism of amyloid plaque formation or the degree of toxicity of the Aβ peptide oligomeric forms[13,14,15]. However, it is known that in addition to the main component of senile plaques, many other proteins can be identified in the formed aggregates. The presence of other components may be caused by their random co-deposition or it can be the result of interactions between Aβ peptide and proteins, which are amyloid plaque components. This event may have twofold significance: it can reduce the concentration of Aβ peptide or its oligomeric forms to protect the body against pathological process[16]. It also can be responsible for the faster amyloid plaque/oligomers formation. Among the proteins found in the amyloid deposits, apolipoprotein E, clusterin, transthyretin, gelsolin, and human cystatin C were identified[17,18]. The presence of hCC is of particular interest due to its described effect on modulations of neuropeptide activity and neurite proliferation[19,20]. The occurrence of cystatin in the deposits was confirmed in blood vessels and brain tissue of people suffering from AD. The biological role and mechanism of interaction between cystatin C and Aβ peptide remains unknown. **In order to understand the role that hCC may play in the process of aggregation of beta-amyloid peptide and/or plaque formation, we have to first determine the binding sites for Aβ-hCC complex. This can be the starting point to new potent inhibitors for plaque formation and potential further AD therapy.**

Human cystatin C and serum amyloid A (SAA)

It was also shown in the literature that cystatin C can form a complex with acute phase proteins and serum amyloid A (SAA)[21]. Naturally occurring amyloid A is a member of apolipoprotein family associated with high-density lipoprotein (HDL). SAA is also responsible for the deposition of insoluble amyloid fibrils (AA) characteristic for rheumatoid arthritis (RA). This chronic inflammation leads to the destruction of the joints ending in a serious disability. As a result of the SAA-hCC complex formation, the SAA loses its ability to induce cytokine production, but the complex has no effect on the physiological function of hCC[21]. Since the formation of this protein complex does not affect the inhibitory function of cystatin C, it may be assumed that the amyloid A binding site in hCC sequence is different from the cysteine protease binding site. Extracellular deposition of amyloid A causes severe complications in the course of RA, and the recognition of binding sites of the SAA-hCC complex may help to solve some problems of patients with rheumatoids diseases. The effect of SAA

binding to hCC on the process of cystatin dimerization and oligomerization has not been investigated so far. **In my work, I examined the binding sites of both proteins, and checked if the amyloid A fragment containing the binding sequence may influence the process of dimerization and oligomerization of human cystatin C.**

Human cystatin C and antibodies

Currently, due to the increasing mobility of people and the blurring of country borders, endemic neurodegenerative diseases cannot be underestimated. An example of such a condition is hereditary amyloid cerebral angiopathy of the Icelandic type, which is caused by the aggregation of the human cystatin C variant. This pathological process is especially severe when a point mutation occurs in position 68 of the hCC sequence (L68Q)[22]. Initially, this genetic mutation was characteristic for the Icelandic population, but currently it commonly occurs in Scandinavian countries, and can be slowly spread over the world[6]. This amyloidosis deserves special attention due to the fact that it affects young people between 20 and 30 years of age[23,24]. Currently, there is no therapy, and no possibility of inhibiting or preventing the pathological aggregation of the cystatin C mutant. Doctors can only relieve general symptoms of the disease. For young people affected by the genetic mutation, every opportunity to develop an effective inhibitor of hCC aggregation or the possibility to use personalized immunotherapy is worth ones weight's in gold.

Professor Grubb with his research group at the University of Lund has shown that catalytic amount of monoclonal antibodies (mAb) directed against human cystatin C is able to significantly decrease the dimerization process, and the aggregation of this protein[25]. In their work, they used murine monoclonal antibodies obtained in their laboratory (IgG2b (k)). In experimental conditions dimerization and aggregation of native cystatin C is induced by the use of promoting factors (guanidine hydrochloride, 37°C). The addition of the antibodies in the ratio 1:10 and 1: 5 (antibody: hCC) to this solution caused the inhibition of the pathological dimerization process[25]. This clearly showed that antibodies can constitute an effective therapeutic able to stop the aggregation of human cystatin C. Initially, only one monoclonal antibody obtained by immunization of animals with the entire hCC molecule, named Cyst13, was commercially available. Currently, the entire set of anti-cystatin C antibodies is on the market (*HyTest*). All of them have been tested by prof. Grubb's group, and it was found that their inhibitory potential significantly varies depending on the clone being tested[26]. They were compared with antibodies produced in the team of prof. Grubb, and now named as HCC3. However, the exact mechanism of inhibition of the pathological process is not known, nor the sequences of epitopes and paratopes involved in the formation of immunocomplexes. **In my**

work, I decided to perform identification of hCC epitopes for selected monoclonal antibodies and determine the hCC key sequences potentially related with inhibition of the aggregation process. In the future, they can be used as targets for the design of inhibitors of cystatin C dimerization and aggregation.

Nowadays, scientists turn their attention to the autoantibodies produced physiologically by the human body, and try to implement this kind of immunoglobulins to immunotherapy of neurodegenerative diseases. Natural autoantibodies (NAbs) are a special type of immunoglobulins produced by the body regardless of the presence of antigen or external stimulation[27]. Their physiological role has not yet been precisely determined. NAbs can be a pathological factor in the body - they are the main cause of some autoimmune diseases; however, they also can have protective functions, which is currently in the main scientific focus. There is no information in the literature about autoantibodies produced in the response to the widespread presence of human cystatin C. **My last goal in the presented work cycle was the detection and molecular characterization of human cystatin C complexes with natural autoantibodies against this protein, found in the pool of immunoglobulins produced by the human immune system.**

Cystatin C is present in all physiological fluids[28] *and in addition to an evident inhibitory function against cysteine proteases, it can interact with various components of physiological fluids. It can affect many processes occurring in the human body. Of particular interest is the interaction with proteins responsible for processes related to the aggregation of human proteins as well as cystatin itself. Identification of the hCC fragments responsible for complexes formation can in future be used as a template for designing effective therapeutics. On the other hand, a map of cystatin C binding sites should be determined to retain the function of protease inhibitor when for example designing inhibitors of its aggregation will be introduced to humans.*

9. The aim of the Scientific Achievement

The main goal of publications presented as the achievement was the identification of the human cystatin C sequences responsible for interactions with biologically active ligands such as peptides and proteins. The basis for the achievement is ten experimental publications that make up the cycle under a common title:

"***Mapping of human cystatin C interactions with biologically active ligands***"**.**

The research work included in the scientific achievement was financed from funds allocated to my person by the Ministry of Science and Higher Education (1264/B/H03/2009/37) and the National Science Center Sonata Bis1 grant (2012/05/E/ST5/03796). The research work was conducted in 2006 - 2017 at

the Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdansk, Laboratory of Mass Spectrometry at the Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk and Department of Medical Biochemistry and Biophysics Karolinska Institutet, Sweden and Laboratory of Analytical Chemistry and Biopolymer Structure Analysis University of Konstanz, Germany.

10. The detailed description of the publications constituting the Scientific Achievement

Human cystatin C and amyloid beta peptide (Aβ)

- **P. Juszczyk** et al. **(2009)** *Binding epitopes and interaction structure of the neuroprotective protease inhibitor cystatin C with beta-amyloid revealed by proteolytic excision mass spectrometry and molecular docking simulation.* **Journal of Medicinal Chemistry (DOI** 10.1021/jm801115e)
- M Spodzieja et al. **(2016)** *Characteristics of C-terminal, β-amyloid peptide binding fragment of neuroprotective protease inhibitor, cystatin C.* **Journal of Molecular Recognition (DOI 10.1002/jmr.2581)**

The research aim:

The aim of these studies was identification of binding sites for the complex of amyloid beta peptide and human cystatin C. The literature data confirming the presence of hCC in amyloid deposits during Alzheimer's disease and inhibition of Aβ aggregation by cystatins indicated that it is reasonable to identify fragments of both molecules involved in the interaction and further characterize this interaction.

Synthetic description of results:

To identify the binding sites, I used epitope excision and extraction techniques combined with the analysis of mass spectra of the obtained fractions[29]. This method is based on the affinity chromatography, where one of the protein/peptide complex partners is attached to a solid matrix and the other one is added to the prepared microcolumn. The formed complex is subjected to enzymatic digestion by selected enzymes (trypsin, AspN, GluC, chymotrypsin, LysC, pronase), and after washing away the released proteolytic fragments that are not involved in complex formation, the complex is dissociated. The obtained factions (supernantant, last ml of wash and elution) are subjected to mass analysis, which allows for quick identification of fragments relevant for the interaction. This is called epitope excision. In the case of epitope extraction, the first step is the digestion of the protein/peptide in the solution by any chosen enzyme, and then complex formation between the protein deposited on the solid matrix and components of the proteolytic mixture. The subsequent steps (removal of unbound fragments and

dissociation of the complex) are identical to those used in excision procedure. Of course, in this case it is possible to cut the binding sequence, which results in a false negative result, however, collective analysis of the results of both approaches gives full information and allows for a quick and precise determination of the binding sequence.

Thanks to the described above approach, the complex of hCC and Aβ was successfully characterized. In the case of the Aβ peptide the binding site is located in the central part of the peptide, residues (17-28). It is a fragment important for the structure and aggregation process of Aβ. It contains the characteristic hydrophobic core, ^{17}LVFFA^{22}, and the residues responsible for the formation of β bend (25-35) arising during the formation of amyloid fibrils. In the human cystatin C sequence, the interaction site is located in the C-terminal part of the protein. Fragment hCC(101-117), which is responsible for binding with Aβ is located in the second loop L2 and the fifth β strand (Figure 1B), and is exposed to the environment and may freely bind the Aβ peptide.

The interaction of the identified fragments in the solution was confirmed by registration of a high resolution mass spectrum (nano ESI FTICR MS) of the complex. Both peptides bind in a 1:1 ratio. Aggregation studies using the full-length Aβ40 and C-terminal hCC cystatin fragment (93-120) and the shortest identified binding sequence, hCC (101-117), confirmed the anti-aggregation potential of human cystatin C.

Structural studies using nuclear magnetic resonance (NMR), circular dichroism (CD) and theoretical techniques such as molecular modeling (MD) of the complex allowed to propose possible spatial structure of complex.

Possibility to use the results:

The molecular characteristics of the hCC-Aβ complex allowed to determine the sites of interaction in the peptide-protein complex. Confirmation of the anti-aggregational potential for the identified C-terminal fragment of human cystatin C allows to start work on the design of inhibitors of the aggregation process (peptides and peptidomimetics) based on the sequence and structure of the identified hCC fragment. This may be important in the search for effective therapeutics in the treatment of Alzheimer's disease.

<u>Human cystatin C and serum amyloid A (SAA)</u>

- M. Spodzieja et al. (2012) *Interaction of serum amyloid A with human cystatin C—identification of binding sites*. **Journal of Molecular Recognition (DOI: 10.1002/jmr.2220)**
- M. Spodzieja et al. (2013) *Interaction of serum amyloid A with human cystatin C – asessment of amino acid residues crucial for hCC-SAA formation (part II).* **Journal of Molecular Recognition (DOI: 10.1002/jmr.2283)**

- M. Maszota et al. *(2015) Structural studies of the C-terminal 19-peptide of serum amyloid A and its Pro -> Ala variants interacting with human cystatin C.* **Journal of Molecular Recognition (DOI: 10.1002/jmr.2457)**

The research aim:

The main goal of the second part of the cycle was the molecular characterization of the human cystatin C complex with the serum amyloid A (SAA) protein. The specific objectives included determination of the stoichiometry of the complex, identification of sites responsible for complex formation, determination of amino acid residues crucial for the interaction, structural studies of protein fragments essential for the complex formation, and determination of the impact of identified fragments on the aggregation processes of both hCC and SAA.

Synthetic description of results:

The use of affinity chromatography technique combined with the enzymatic digestion of the hCC-SAA complex and fraction analysis using mass spectrometry as a basic approach allowed the determination of sequences of both proteins responsible for their interaction. In this case, the proteins were interchangeably bound to the sepharose beds, and the second partner of the complex was used i) for complex formation and next digested in epitope excision protocol; ii) digested in solution and then complex formation between protein and proteolytic peptides in epitope extraction approach. In the case of digestion of human cystatin C (in solution and in complex with SAA) results revealed the C-terminal fragment of hCC as responsible for interaction with serum amyloid A. Based on the conducted studies, the critical amino acid residues for the interaction of the hCC fragment (96-102) are: serine at position 98 and tyrosine at position 102. Both of these amino acids have a hydroxyl group in the side chain, and may thus act as both donor and acceptor of the hydrogen atom during the formation of the protein complex.
In the case of SAA protein, a C-terminal fragment of the protein (86-104) was identified as the one involved in the formation of the complex with human cystatin C. Amino acid residues critical for the interaction are as follows: arginine at position 96, lysine at position 90 and lysine at position 103. They all have a basic character and in the experimental conditions have a positive charge on the nitrogen atoms of the side groups. Replacing one of them with alanine results in the loss of capability for binding to human cystatin C. They may act as hydrogen donors in the formed protein complex. In order to confirm the interaction of both fragments, the ESI-FTICR MS and MS/MS spectra for mixed synthetic SAA(86-104) and hCC(93-102) fragments were registered. They proved the formation of a peptide complex in solution and showed that the peptides interact with each other in a 1:1 stoichiometric ratio.

Using techniques such as electrophoresis and molecular filtration, I attempted to determine the influence of identified binding fragments on their aggregation processes. Obtained results showed that SAA in phosphate buffer at 37^{o}C revealed tendency for aggregation and association. The protein, like its (86-104) fragment responsible for interaction with hCC, does not inhibit the dimerization/oligomerization of human cystatin C. The effect of hCC on SAA aggregation was not clear.

Structural studies of the hCC(96-102) fragment, which in the native protein is located in the fourth β-strand, is characterized by a disordered structure with a small proportion of β-sheet structures. This peptide is hardly soluble in aqueous solutions, and addition of trifluoroethanol (TFE) does not improve its solubility. Due to the presence of a cysteine residue at position 97, it forms covalent dimers. The SAA fragment (86-104) has three proline residues in its sequence (positions 92, 95, and 101) that affect its structure. In aqueous solutions the unordered structure dominates, and the α-helical structure can be forced only by replacing one of the three proline residues with an alanine. Critical in this case is position 97. This peptide does not tend to associate or aggregate. It is worth to mention that it undergoes spontaneous pH dependent autohydrolysis. In an pH 7.4 or slightly acidic (pH 6) hydrolysis takes place both from the N- and C-terminus, and this process is not observed in alkaline solutions. The bond between the Asp91 and Pro92 residues is particularly susceptible to this process. The biological significance of this process and the possible role of human cystatin C in the hydrolysis of the C-terminal fragment of SAA has not yet been elucidated.

Possibility to use the results:

The presented work shows that the serum amyloid A binding site is, similarly to the Aβ peptide, located in the C-terminal part of the cystatin C. Comparison of the sequence of binding sites in cystatin C for the tested proteins will give us the opportunity to further specify the hypothesis about the special role of this fragment of the protein.

Human cystatin C and antibodies

- A. Śladewska et al. (2011) *Identification of the epitope for anti-cystatin C antibodies (Cyst-13).* **Journal of Molecular Recognition (DOI: 10.1002/jmr.1100)**
- I. Behrendt et al. **(2016)**. *Epitope location for two monoclonal antibodies against human cystatin C, representing opposite aggregation inhibitory properties.* **Amino Acids** (DOI: 10.1007/s00726-016-2242-z)

- M. Prądzińska et al. **(2016)**. *Application of amide hydrogen/deuterium exchange mass spectrometry for epitope mapping in human cystatin* C. **Amino Acids (DOI: 10.1007/s00726-016-2316-y),**
- M. Rafalik et al. (2018) *The identification of discontinuous epitope in the human cystatin c – monoclonal antibody hCC3 complex*. **Journal of Proteomics** (**DOI**: doi.org/10.1016/j.jprot.2018.04.020)

The research aim:

The aim of the work collected in the publications presented above was the molecular characterization of human cystatin C complexes with monoclonal antibodies. Three of them are commercially available clones (*HyTest company*) designated as: Cyst10, Cyst13 and Cyst28. HCC3 antibody was received from prof. Anders Grubb from University of Lund, Sweden[30]. All of the antibodies have been previously tested for the inhibition of the human cystatin C dimerization. mAb Cyst10 has the lowest impact on hCC dimerization, while the other three antibodies have different inhibitory potential: HCC3 - 60%, Cyst13 - 22%, Cyst28 - 75%[26].

Synthetic description of results:

I carried out the epitope identification using the epitope excision/extraction mass spectrometry technique. It allowed the unambiguous determination of the epitope for the Cyst13 clone as linear and located in the C-terminal part of the protein, residues (107-114). In the case of other antibodies, this technique indicated the discontinuous nature of the epitopes and allowed for the selection of several fragments for potential epitopes. Therefore, as a method verifying the obtained results, we used a proton/deuter exchange approach combined with mass spectrometry analysis.

HDX experiments were conducted in two independent laboratories (clones Cyst10 and Cyst28 were tested in Karolinska Institute, Sweden and antibody HCC3 in IBB PAS in Warsaw). In both cases we managed to confirm some of the identified epitope fragments, but also discrepancies appeared. The summary of the received results is presented in Table 1.

Table 1. *Comparison of human cystatin C epitopes identified by extraction/excision MS techniques and HDX MS technique*.

mAb	E/E MS	HDX-MS	hCC structure
HCC3	--	17-28	α–helix
	54-64	52-62	L1
	93-103	100-105	β4
Cyst10	-	53-61	L1
	60-70	-	β3
	96-102	-	β4
	101-111	101-112	β4–L2–β5
Cyst28		41-48	β2
	53-62	53-61	L1
	-	65-73	β3
	85-91/92-99	81-99	β4
	101-111	-	β4–L2–β5

E/E MS – epitope extraction/excision mass spectrometry
HDX MS – hydrogen/deuter exchange mass spectrometry

In the case of Cyst10 and Cyst28 antibodies, the characterization of the antibodies alone was performed by: recording intact mass spectra, which revealed differences in the mass of both clones; the analysis of carbohydrate residues attached to the antibodies; determination of subclasses: Cyst28 belong to the IgG1 subclass, and Cyst10 to IgG3. The use of microscale thermophoresis (MST) allowed to determine and compare dissociation constants for both clones. For the hCC-Cyst28 complex, the dissociation constant takes a value 20.2 ± 1.85 nM, and for hCC-Cyst10 - 141 ± 13.2 nM.

Possibility to use the results:

The identified epitopes indicate the location of the structural elements of human cystatin C, which may become targets for the design of inhibitors of the dimerization and aggregation process of hCC. The second step will be the paratope identification which will give us a full picture of immunocomplexes of human cystatin C.

- M. Prądzińska et all. (2016). *Isolation and characterization of autoantibodies against human cystatin C.* **Amino Acids** (DOI 10.1007/s00726-016-2271-7)

The research aim:

The main purpose of the last work from the presented cycle was the detection and characterization of human cystatin C complexes with natural autoantibodies (NAbs) against this protein, found in the pool of immunoglobulins produced by the human body. The work carried out by prof. Grubb using monoclonal antibodies against human cystatin C gave very promising results on the *in vitro* inhibition of the hCC dimerization process.

Before proceeding with the design of immunotherapy, it is important to check whether the human body is able to produce autoantibodies against hCC, determine, if possible, an epitope and paratope sequences for this immunocomplex, and importantly check the potential of NAbs to inhibit dimerization and aggregation process of cystatin C and its L68Q mutant. In addition, the obtained results may in the future contribute to broadening of the knowledge on the interaction of autoantibodies with human cystatin C.

Synthetic description of results:

To check the presence and further isolate the autoantibodies against human cystatine C from the IgG fraction, again affinity chromatography was used. Prepared column with hCC attached to the sepharose allowed for the isolation of NAbs antibodies, which constitute 0.2-0.3% of the total IgG fraction. The purity of the isolate was checked chromatographically (HPLC and SEC) and using one and two dimensional gel electrophoresis. I confirmed that the obtained NAbs sample contains only polyclonal antibodies represented by subclasses: 78% of IgG1, 15% of IgG2, 6% of IgG3 and 1% of IgG4. The use of mass spectrometry allowed the identification of carbohydrate residues attached to autoantibodies from the IgG1 and IgG2 subclasses: mainly di-sialylated, bi-antennary complex-type N-glycan with one galactose attached to the either end of the antennae FA2G1; di-sialylated, bi-antennary complex-type N-glycan FA2 and di-sialylated, bi-antennary complex-type N-glycan with two galactose attached to the either end of the antennae FA2G2.

Before the experimental identification of epitopes, a theoretical analysis of the human cystatin C sequence for fragments presenting the antigenic character was carried out. We used free IEDB websites (http://www.iedb.org/) with the algorithms available there: *EliiPro*, *Bepipred*, and the *Kolaskar and Tongaonkar* algorithm. The obtained results indicated that the entire hCC sequence may contain fragments of different lengths, which can be both linear and discontinuous epitopes.

For the experimental identification of epitopes for isolated autoantibodies, we used, as in the case of monoclonal antibodies, the epitope excision and extraction technique coupled with mass spectrometry, and a proton/deuter exchange interaction technique coupled with mass spectrometry and additional techniques such as affinity chromatography and plate enzyme immunoassays. In the case of epitope excision and extraction, autoantibodies were immobilized on a solid matrix, and cystatin C was subjected to digestion. Immunocomplex was created with the whole molecule and then subjected to digestion (trypsin, AspN, pronase; epitope excision), or the protein was digested in solution and the complex was created between autoantibodies and hCC proteolytic fragments. The carried out experiments indicated three hCC fragments, which may be considered as epitopes for anti-hCC NAbs. Two of

them are located in the L1 loops (fragment 53-62) and L2 (fragment 101-115), and one is in the β3 strand (fragment 92-99). HDX analysis, where the immunocomplex was created in the solution, indicated residues 41-48 and 65-73 as recognized by NAbs. Both techniques confirmed the epitopes to be: a fragment located in the middle part of cystatin C (53-62) and fragments from the C-terminal part (92-99, 96-102, and 101-115). However, the interaction with the autoantibodies of the fragment from the N-terminus of hCC and the fragment located in the α-helix identified by HDX MS were not confirmed.

An attempt to determine the effect of isolated autoantibodies on hCC dimerization did not give a definite result and requires further experiments. Anti-hCC NAbs caused dimerization of hCC immediately after mixing, but after 3 days of incubation the amount of dimer in the control and the examined sample was the same. This process should be further investigated.

Possibility to use the results:

This is the first and till now the only work devoted to autoantibodies against human cystatin C. Learning the sequence and structure of the epitope and paratope for NAbs will in the future be the basis for further work on designing effective inhibitors of the human cystatin C aggregation process. Based on current studies, it has been proposed that naturally occurring autoantibodies, by interacting with a physiologically important and simultaneously neurotoxic protein, eliminate them from the systemic circulation before it reveals its second - toxic nature. However, the way in which antibodies inhibit the formation of aggregates themselves is not known. NAbs can also be used in the diagnosis of the early stages of the disease. This kind of diagnosis can be used in autoimmune diseases such as rheumatoid arthritis, lupus erythematosus, or type 1 diabetes[31]. These diseases are characterized by a long preclinical phase during which the developing disease can be diagnosed on the basis of the level of appropriate autoantibodies. A similar approach can be used for neurodegenerative diseases whose progression depends on the duration of the disease. There were differences in the level of autoantibodies in healthy and sick subjects[32,33]. Promising results of autoantibody therapy and their diagnostic potential, combined with an incompletely elucidated mechanism of action, tend to expand the collection of examined amyloidogenic proteins and the neurodegenerative diseases caused by them in this context[34,35,36]. Therefore, we wanted to isolate NAb-hCC complexes, subject them to molecular characterization and examine their impact on the cystatin C self-association process. Thanks to that, it will be possible to not only learn the mechanism of their action, but also its reference to other neurodegenerative diseases. The development of new therapeutic methods and diagnostic tests for HCCAA,

based on the autoantibodies present against the hCC in the serum, is another forward-looking consequence of the implementation of the project.

Summary:

While comparing the obtained results of identification of binding sites for human cystatin C complexes with:

- Amyloid beta peptide Aβ40,
- Serum amyloid A,
- Monoclonal antibodies:Cyst10, Cyst28, Cyst13 and HCC3,
- Autoantibodies anti hCC,

it can be concluded that the entire hCC sequence may be involved in the interaction with ligands (Table 2). Only short fragments from the N- and C-terminus (residues 1-16 and 118-120), as well as fragment 70-80 are excluded from this interaction (Figure 2). The N-terminal fragment is characterized by considerable mobility and lack of a specific structure, and it may be cleaved in vivo from the native protein (first 10-11 residues) by the action of neutrophil elastase[37,38]. Fragment 70-80 may be excluded due to the fixed structure introduced by the disulfide bridge formed by the cysteine residues 73 and 83.

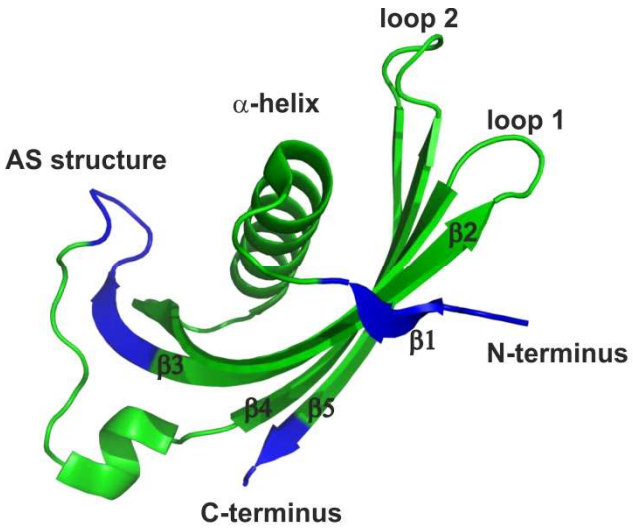

Figure 4 *Structure of human cystatin C (PDB 3GAX): marked in blue are fragments not involved in the interaction with ligands, while green indicates the cystatin fragments capable of interacting with proteins and peptides.*

Table 2. *Fragments of human cystatin C identified in the course of the work as responsible for the formation of complexes with tested proteins and peptides.*

Cystatin C fragment	Ligand	Experimental method
1-16	-	-
17-28	HCC3	HDX MS
41-48	Cyst28	HDX MS
52-64	HCC3, Cyst28	EiE MS, HDX
60-70	Cyst10	EiE MS
70-80	-	-
81-99	Cyst28	E/E, HDX MS
93-103	HCC3	E/E MS, HDX MS
96-102	SAA	E/E MS
101-111	Cyst10 Cyst28	E/E MS, HDX E/E MS
101-117	Aβ	E/E MS
107-114	Cyst13	E/E MS
117-120	-	-

E/E MS – epitope extraction/excision mass spectrometry
HDX MS – hydrogen/deuter exchange mass spectrometry

11. The main results of this Scientific Achievement

The most important accomplishments of this Scientific Achievement are:

- ***Introduction of a new research topic and new research methodology to the Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdansk***

  The works carried out so far in the Department of Biomedical Chemistry related to human cystatin C were associated with structural studies, elucidation of the mechanism of dimerization and aggregation of hCC, and the search for inhibitors of this process. Until I submitted a grant project proposal to the Ministry of Science and Higher Education regarding the search for biologically active ligands of human cystatin C, no research into protein complexes had been undertaken in my Department. The studies of interactions between the complex components were largely based on the epitope search using MS-assisted limited proteolysis. The applied epitope excision and extraction mass spectrometry approach, which was the basis of my postdoctoral internship at the University of Konstanz, was then permanently introduced to the Department members workshop.

- ***Molecular characterization of human cystatin C complexes with amyloid beta peptide and serum amyloid A***

  My/our studies resulted in the first characterization of the interaction of human cystatin C with the amyloidogenic peptide and the amyloidogenic protein. The results confirmed that in addition to the protease inhibitor function, hCC may also participate in processes related to the aggregation of

other molecules, which, as in the case of interactions with Aβ, may have neuroprotective potential.

- ***Identification of epitopes for monoclonal antibodies directed against human cystatin C***

  As part of the studies on human cystatin C immunocomplexes, epitopes for three commercial monoclonal antibodies Cyst10, Cyst13, and Cyst28 and the HCC3 mAb obtained for research through collaboration with the University of Lund, were identified.

- ***Isolation from IgG fraction, epitopes identification and characterization of natural autoantibodies against hCC***

- ***Isolation of natural autoantibodies against hCC from human IgG fraction, their identification and characterization***

  The work carried out in this part allowed for the first time to isolate and characterize autoantibodies directed against human cystatin C. The ability of the human immune system to produce immunoglobulins against hCC has not been reported previously in the literature. The studies were conducted as part of the NCN Sonata Bis grant.

12. New research plans directly related to the subject of my Scientific Achievement

In my further research I intend to continue the studies of complexes of human cystatin C with the main components of biological fluids. For this purpose, I intend to use the experience that I gained in the field of mass spectrometry and the knowledge gained from previous work.

In the course of work related to the identification of epitopes, it turned out that one fragment of cystatin C presents surprising properties. Currently, I am continuing the work to determine its potential in creating complexes with other proteins and to determine the application potential of this peptide.

In 2014, I became the head of the Laboratory of Mass Spectrometry created as part of the Specialist Laboratories of the Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk. It allowed me to develop my interests related to mass spectrometry, improve my work skills and develop my own research topic based on this technique. Much of my academic work will also be related to participation in projects conducted at IFB and cooperation with Polish and foreign scientists conducting research requiring the use of proteomic analyzes. Besides, very important task of my work will also be the development of the Laboratory itself, raising funds for new instruments and good maintainance of the existing equipment.

13. Presentation of other Scientific and Research Accomplishments

(The detailed list of all my research publications (IF, Ministerial Publication Points and number of citations per article) is presented in the Attachment no. 5 together with the list of all my other achievements.)

I started my scientific career in 1996, when I became a student of the first year of chemistry at the Faculty of Chemistry of the University of Gdańsk. In the third year of studies, after completing the course of organic chemistry, I applied to the head of the Department of Organic Chemistry, prof. Zbigniew Grzonka, who accepted me to his research group. I did my master's thesis under the supervision of dr hab. Aleksandra S. Kołodziejczyk, who infected me with love for learning, especially organic chemistry. The first works performed within the specialization laboratory concerned the preparation of amino acid derivatives used in the synthesis of peptides and peptidomimetics. At that time I got acquainted with the secrets of liquid chromatography, which I learned from prof. Franciszek Kasprzykowski. The subject of my master's thesis was "*Synthesis of chiral 1,2-diamines with an amino acid skeleton using the Mitsunobu reaction*". It concerned the reaction between the hydroxyl group of the substrate and the suitable nucleophilic agent in the presence of triphenylphosphine and ethyl (DEAD) or isopropyl (DIAD) ester of azodicarboxylic acid. My task as a graduate student was to obtain substrates, specifically chiral protected diamines, which were successfully used to perform the final reaction, and the results were presented in the form of publication[39]. In addition, I investigated the possibility of using the Vitride reagent to obtain chiral amino alcohols from amino acids, and the results are described in the next publication[40].

In 2001, I became a PhD student at the Faculty of Chemistry. The promoter of my doctoral thesis was prof. Zbigniew Grzonka, and the direct scientific care was exercised by dr hab. Aleksandra S. Kołodziejczyk. For four years I have studied the aggregation of beta amyloid peptide, which is responsible for the formation of amyloid deposits in people affected by Alzheimer's disease. Of my particular interest were hereditary variants of this disease, which are associated with the occurrence of point mutations in the Aβ sequence. These mutations affect the aggregation process, mainly by accelerating the formation of amyloid fibrils. In the course of my work, I managed to determine for the model peptide Aβ (11-28) the potential mechanism of aggregation, in which the formation of a transitory helical state is crucial. I carried out structural and aggregation research, and the results of the work were published in the form of two publications, one during PhD studies; the second one was published after completing the study[41].

In addition to the main subject, I also took part in cooperation with national and foreign research centers, which were carried out by prof. Grzonka for many years. This included cooperation with prof. Henryk Kozłowski from the University of Wrocław, prof. Roman Kaliszan and prof. Tomasz Bączek from Gdańsk Medical University and prof. Falk Fahrenholz from the University of Mainz. My role was mainly focused on the synthesis and purification of peptides that served for further scientific research. I participated in the preparation of manuscripts that were the result of these collaborations. In 2005 I took my first postdoctoral internship with dr Maria Gaczyńska from the University of Texas Health Science Center in San Antonio, Texas. For many years, Dr. Gaczyńska's group has been researching the proteasome. During 12 months of the internship I was involved in the design, synthesis, purification and structural studies of proteasome inhibitors based on the structure of peptide PR11. In 2006 I took a postdoctoral internship with prof. Michael Przybylski, who led the analytical laboratory at the University of Konstanz in Germany. Prof Przybylski is the world-class specialist in the field of mass spectrometry, and his scientific interests are also focused on Alzheimer's disease. It was a great opportunity for me to train the application of mass spectrometry in proteomics research and to continue the subject of my doctoral thesis. For the first three months, I worked with Dr. Gabriele Parashiv, who was involved in identification of epitopes and paratopes for immunocomplexes of Aβ peptide with nanobodies directed against the Aβ40 peptide. This allowed me to become proficient in using the techniques of epitope excision and extraction and to master the operation of mass spectrometers. After finding the first information on the influence of human cystatin C on the aggregation of Aβ peptide in the literature, I decided to use the newly acquired experience to work on the identification of binding sites in the Aβ-hCC complex. I conducted the work in cooperation with prof. Grzonka and dr Aneta Szymańska from the Department of Medical Chemistry, University of Gdańsk and prof. Przybylski and Dr. Parashiv. After several months of hard work, we managed to prepare a manuscript, which was published in the Journal of Medicinal Chemistry. In addition, the results obtained have been incorporated into the patent application: Patent Application No. 08002878.0-2405: “Neuroprotective peptides and paratopes recognizing a sequence-specific β-amyloid epitope for diagnosis and therapy of neurodegenerative diseases” (European patent).

After completing my internship in Konstanz, in 2007 I returned to Gdańsk and got a job at the Faculty of Chemistry of the University of Gdańsk. I continued my academic work in the group of prof. Zbigniew Grzonka where I took care of my own research topic. I dealt with the search for biologically active ligands for human cystatin C. In 2009, I obtained the first grant of the

Ministry of Science and Higher Education to carry out my own research. This allowed me to conduct research on human cystatin C complexes with amyloid A plasma protein and the first commercially available anti-hCC antibody, Cyst13 clone. In the research, I was supported by Anna Śladewska, MSc and Marta Spodzieja, MSc who carried out their doctoral studies under my scientific supervision. They are the first PhD students who have been gaining scientific experience under my scientific supervision, and currently both of them continue their individual academic work: Dr. Anna Śladewska at the University of Konstanz, and Dr. Marta Spodzieja at the Faculty of Chemistry of the University of Gdańsk. The results of our joint research have been published in the form of four experimental works.

With time more commercially available anti-hCC antibodies appeared on the market. Moreover, our co-worker from the University of Lund, prof. Anders Grubb published the work on comparison of inhibition potential for series of anti-hCC antibodies. This prompted me to continue the studies and identify hCC fragments whose shielding during immunocomplex formation results in inhibition of dimerization process. I became a co-supervisor of the thesis of three PhD students involved in these studies (mgr Martyna Prądzińska, MSc, Izabela Behrendt, MSc, and Monika Rafalik, MSc), after it was officially allowed.
In 2012, I obtained research funding under the NCN Sonata Bis grant for work related to isolation and characterization of autoantibodies against human cystatin C, which I carried out with Martyna Prądzińska, MSc. For the first time we managed to isolate and characterize autoantibodies against hCC. Thanks to the technique learned in Konstanz: limited proteolysis coupled with mass spectrometry, we were able to determine the hCC epitopes for isolated polyclonal autoantibodies.
In 2013, I also started the third postdoctoral internship at the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk. At that time, prof. Krzysztof Bielawski (the PI of the project) was looking for a specialist in the field of mass spectrometry as part of the Mobi4Health (FP7) project. As a result of the recruitment, I got a job as a Post-doc and the manager of the new Laboratory of Mass Spectrometry. My task was to organize a new laboratory from scratch. It gave me an opportunity to pursue my interests related directly to the MS technique and to demonstrate my position as a laboratory manager. Participation in the Mobi4Health program gave me an opportunity to attend training at the headquarters of the Sciex company (Darmstadt, Germany) in the field of handling and conducting proteomics on spectrometers: QTRAP 6500, TripleTOF 5600+, and MALDI TOF/TOF 5800. I participated in the organization of four MS trainings conducted by Sciex at IFB. Together with Dr. Katarzyna Macur in 2016, we

prepared and conducted workshops on basic proteomics for scientists from the Tri-City.

In 2016, after completing the Mobi4Health project, I obtained further employment at the Faculty of Biotechnology, where I work to this day. Together with Dr. Katarzyna Macur, I run, as part of the Core Facility Laboratories, the Laboratory of Mass Spectrometry, in which, in addition to carrying out our own projects, we conduct research supporting all scientists from the University of Gdańsk and other universities. The laboratory also performs analyzes for scientists from all over the country, which greatly expands my scientific horizons. We also establish new cooperations with mass spectrometry specialists. With prof. Ciborowski from the University of Nebraska we are preparing next workshops devoted to basic proteomics techniques, which will include both lecture and practical classes, and it will be provided in May 2018.

During my scientific work I also conducted didactic classes. Since the time of my doctoral studies I have conducted laboratory classes in organic chemistry and organic analysis. After obtaining employment at the Faculty of Chemistry, I continued to conduct laboratory classes in organic chemistry, analysis of biologically active compounds, and I was supervising specialization and master's theses. At a later stage, I prepared a monographic lecture for second-degree students: “Selected problems in peptide chemistry” and participated in the development of laboratory classes from the Advanced Chemistry Laboratory for second-degree students. Together with dr hab. Aneta Szymańska I carried out didactic classes in the form of laboratory exercises from Organic Chemistry: “Basic laboratory techniques” as a part of the project “Personnel training for an innovative knowledge-based economy in the field of agrochemistry, chemistry and environmental protection (Inno'AgroChemOś)”. I have developed original didactic lecture: “Why chemical reactions are running?”.

Currently, I intend to devote myself to the preparation of practical classes focused on mass spectrometry and basic proteomics techniques, which are now used as the basis for research.

I also consider as my great success the reconciliation of my academic work and motherhood. In 2010, I became a mother for the first time - daughter Maria, and in 2012 for the second time - son Adam. It is not easy to reconcile two such different worlds, but thanks to the family's support I managed not to give up the scientific part of my life.

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