Summary of Professional Accomplishments

1. Name
2. Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation
3. Information on employment in research institutes or faculties/departments or school of arts
4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act
5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions
6. Presentation of teaching and organizational achievements as well as achievements in popularization of science or art
7. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.

Piotr Hamerla
.....
(Applicant's signature)

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The summary of professional accomplishments contains information about the career path, employment in national and international scientific institutions. The habilitation applicant describes also the publications included in the habilitation cycle entitled "**Laser spectroscopy methods for detection of aggregated proteins and peptides doped with fluorescent markers**".

Summary of professional accomplishments

In 2009 I obtained the master engineer degree in biotechnology of Wrocław University of Science and Technology, Chemistry Department with specialization "molecular nano and biophotonics for telecommunications and biotechnologies" and a master of physics of French Ecole Normale Superieure de Cachan in 2010. After finishing the MSc studies I accomplished three months scientific internship at Australian National University in Canberra.

Next, I moved to Sweden where I did PhD studies at Chalmers University of Technology in Gothenburg. In a bilateral agreement I was also a PhD student of Wroclaw University of Technology. During the PhD I was on a one month internship at the University of California, Berkeley. I also participated in many international conferences where I was presenting posters or was a speaker (e.g. in RSA, Mexico, France and Poland). The dissertation of PhD thesis at Chalmers University of Technology was in 2013 and at Wroclaw University of Technology it was in 2014.

After the PhD studies I spent two years at the University of California, Santa Barbara in Nobel laureate research group, prof. Alan Heeger. In 2016-2017 I worked as a researcher at Chalmers University of Technology in Sweden. After one year I moved to Poland, where I was employed as an assistant professor (adiunkt position) at the Institute of Physical Chemistry, Polish Academy of Science (IPC PAS). In IPC PAS I worked for six months.

From August 2018 I'm employed as an assistant professor (adiunkt position) at the University of Warsaw, Physics Department, Optics division in Maria Skłodowska-Curie reintegration grant. I'm working on implementation of laser optics methods for detecting conformational changes in proteins linked with neurodegeneration diseases.

My publication list consist of 19 articles whereby in case of 14 I'm the first author. All of them were published in the leading international journals in area of physics and physical chemistry referring to research on important biological and medical aspects.

In the protein aggregation process β-sheet structures are formed which are the dominating structural motif in amyloid fibrils. The amyloid fibrils are responsible for development of neurodegeneration diseases such as Alzheimer's or Parkinson's. My first publication regarding application of laser technology for amyloid fibrils detection was published in prestigious Nature Photonics journal.

The article was published in 2013 and was included in my doctoral thesis. It is also included in the cycle of scientific articles for habilitation degree because it initiated my work on application of laser spectroscopy and nonlinear optics to detect erratic protein structure linked to neurodegeneration. This article is also unrelated with the main focus of doctoral thesis which was based on cycle of 6 scientific publication, whereby 5 articles were about spectroscopic analysis of DNA structure and DNA interactions with intercalators. The publication in Nature Photonics is the effect of shifting my research interest from areas which I was exploring during PhD studies under the supervisors guidance. It is the beginning of my research independence.

Presented cycle of scientific articles [1-9] is the effect of research activates performed in years 2013-2019 and is a scientific work entitled "Laser spectroscopy methods for detection of aggregated proteins and peptides doped with fluorescent markers".

At first the cycle of scientific articles is described in chronological order starting from publication from 2013. Next the cycle is is divided into two sections whereby I present the methodology, the direction of my research and process of accomplishing the work presented in the habilitation cycle and summarized with article from year 2019 [9].

In publication [1] I investigated nonlinear absorption properties of proteins in aggregated and non-aggregated form. In the article I showed strong correlation between protein structure and nonlinear absorption enhancement. As an explanation of nonlinear absorption enhancement I proposed intermolecular interactions between the aromatic amino acids, tyrosine, phenylalanine and tryptophan. The distance between the aromatic amino acids in the aggregated protein is of 3-4 Å what leads to cooperative effects and nonlinear absorption enhancement.

In publication [2] I investigated the interactions of two enantiomer forms of ruthenium metal-organic compounds with amyloid fibrils. Ruthenium compounds were chosen for investigation because of large nonlinear absorption cross sections and optical phenomenon called "light switch effect", which means that in presence of hydrophobic biomacromolecules their fluorescence quantum yield increase significantly. My research revealed that one of the enantiomers has better steric confinement in channels of the β-sheet at the fibrils surface. As a result of the better adjustment to amyloid channels, the enantiomer exhibits greater fluorescence quantum yield than the other studied form. In consequence of greater fluorescence quantum yield it is possible to detect the bound form of the fluorophore to amyloid fibrils using two-photon excitation.

In publication [3] I presented the concept of using amplified spontaneous emission to detect amyloid fibrils. By the optical pumping in the dye absorption band of the bound form of Stilbene 420 to amyloid fibrils it exhibits stimulated emission evolving to random lasing. The random lasing spectra were dependent on amyloid fibrils structure.

Publication [4] was about the interactions of polyfluorene with amyloid fibrils. Polymers are conjugated long chain molecules with multiple bonds. Such structure cause sizeable enhancement of two-photon absorption. Simultaneously, conjugated polymers have good quantum yield of fluorescence which makes them attractive molecules for amyloid fibrils staining. Polymers can be detected using multiphoton optical techniques.
As I showed in the article, in result of interactions with amyloid fibrils, polymers aggregated at the fibrils surface what caused additional intermolecular enhancement of nonlinear absorption. However, due to

polymer aggregation and charge transfer between the amino acids in amyloid fibrils and polymer chains, strong fluorescence quenching occurred.

In publication [5] I investigated Rhodamine 6G in presence of two proteins, insulin and lysozyme, which in denaturation conditions form amyloid fibrils of different morphologies. I demonstrated the correlation between protein aggregation and fluorophore aggregation that is bound to the amyloid fibrils surface. Therefore, the rate of fluorophore aggregation affects the threshold of amplified spontaneous emission generation. In article conclusions I proposed that generation of amplified spontaneous emission in presence of protein aggregates depends on three factors: fluorescence quantum yield of specific fluorophore aggregates bound to amyloid fibrils, the rate of fluorophore aggregation and scattering yield of distinct structural form of protein aggregates. The conjugation of that three parameters allows to understand and explain the optical phenomenon of amplified spontaneous emission in fluorophores mixed with proteins and protein aggregates.

In publication [6] the optical phenomenon of amplified spontaneous emission in Rhodamine 6G, was used to detect amyloid oligomers which are formed during the first stage of protein aggregation, so called the nucleation phase. In that work I compared results of spontaneous emission with results of amplified spontaneous emission of the same protein-dye samples. Only the generation of amplified spontaneous emission allows for achieving appropriate sensitivity required for differentiating early forms of protein aggregates from native proteins or mature fibrils.

In publication [7] I used ultrafast femtosecond spectroscopy to analyze the excited states of Fluorescein dye bound covalently to the N-termini of the peptide. The peptides used in the experiments form various configurations of alignment of the β-sheets in respect to each other. The fluorophore attached to the N-termini followed the alignment of the β-sheets in the peptide aggregates, which organize itself in the so called steric zipper structure. Such a research approach allowed for analysis of the Fluorescein excited state dynamics in relation to the β-sheet organization in the steric zipper structure. To investigate the excited state dynamics of the Fluorescein in the peptide aggregates, time-resolved spectroscopy and in particular pump-probe technique was employed. Due to the fluorophores coupling related to the short interspace distance in aggregates (few angstroms), the excited state dynamics was dependent on fluorophore organization in the steric zipper structure. The relaxation from the excited state last from few hundreds femtoseconds up to few tens of picoseconds. Based on the results, I concluded that application of ultrafast spectroscopy to examine the relaxation processes can be used for optical detection of various spatial alignments of β-sheet in the steric zipper structures.

In publication [8] I used popular polymer, polithiophene and time-resolved techniques to prove that main reason for fluorescence quenching in polymers interacting with amyloid fibrils is related to the intrachain twisting which cause loose of bond conjugation. This observation was confronted with popular amyloid fluorescent marker i.e. Thioflavin T. Thioflavin T belongs to a group of compounds named molecular rotors. In effect of interactions with amyloid β-sheets the rotation of aromatic rings becomes inhibited. In result, Thioflavin T exhibits strong fluorescence enhancement due to inhibition of non-fluorescent twisted internal charge transfer (TICT). In Thioflavin T bound with amyloid fibrils the relaxation occurs from locally excited state (LE). The comparison of two fluorophores, polythiophene polymer and Thioflavin T dye, showed that the binding mechanism with amyloid fibrils is the key to achieve high fluorescence quantum yield. In result of interactions with amyloid fibrils, in polymers strong fluorescence

quenching occurs which is related with polymers aggregation, whereby in dyes the binding mechanism can improve the quantum yield of fluorescence.

In publication [9] I investigated interactions of Coumarin 307 dye with lysozyme protein in native and aggregated state. I employed two-photon excitation in near infrared spectral range to generate amplified spontaneous emission. The results presented in the article showed that two-photon excited amplified spontaneous emission preserve high rate of sensitivity for detection of amyloid fibrils. This approach allows to detect aggregates of various morphologies and examination of the samples at different stage of protein aggregation using less invasive methods based on nonlinear optics and two-photon excitation in near infrared.

The habilitation cycle is composed of two sections. First part is based on articles [1, 2], [4], [7], [8]. It refers to the research on employing different laser spectroscopy methods for analysis of amyloid fibrils interactions with various types of organic fluorescent markers. Five different type of amyloid fibril markers were examined: natural aromatic amino acids incorporated in the protein sequence (tyrosine phenylalanine and tryptophan) [1], metal-organic compounds [2], polymers [4, 8], fluorophores attached covalently to the N-termini of the peptide [7], and fluorophores interacting electrostatically with amyloid fibrils [8]. The results of that research work showed that photophysical properties of the markers depends on amyloid structure and the mechanism of interactions. Based on the obtained results the group of organic fluorophores binding electrostatically to amyloid fibrils was most promising for application the laser optics and optical phenomenon of amplified spontaneous emission for detecting protein aggregation.

Publications [3], [5, 7], [9] are assembled as a second part of the cycle. Fluorophores Stilbene 420, Rhodamine 6G and Coumarin 307 were examined as potential amyloid fibril markers. Three mentioned fluorophores are also lasing dyes. Their good fluorescence quantum yield was used in experiments on amplified spontaneous emission, stimulated emission and random lasing in presence of protein aggregates. The aim was the examination of the detection sensitivity based on optical phenomenon of amplified spontaneous emission and investigation the detection of amyloid fibrils by possibly least invasive methodology using techniques based on nonlinear optics.
The near infrared region is named a biological window whereby water has the lowest absorption coefficient and light can deeply penetrate the tissue without destructing the biological material. It is highly appreciated technology in research on biological system and in diagnostics. Simultaneously, methods having high rate of sensitivity are developed. In second part of the habilitation cycle I present the possibility of applying two-photon excited amplified spontaneous emission in near infrared region for effective detection of amyloid fibrils.

Publication [1] was prepared close to the end of my PhD studies and was the introduction to the research on protein aggregation. After the PhD dissertation at Chalmers in Sweden and at Wrocław University of Technology in Poland, I received prestigious grant from Swedish Research Council (VR) and research project from Chalmers Area of Advance for continuing the research on detection of amyloid fibrils by optical methods. As a result of the two grants six articles were published [2, 3, 4, 5, 6, 7]. In years 2017-2018 I accomplished the research project funded by the International Brain Research Organization (IBRO). As an outcome of the IBRO project, one article was published [8]. I'm recently a principle investigator in Maria Skłodowska-Curie grant funded by FP7 framework programme of European Union. In time of MSCA project, one article was published [9]. Both fellowships, IBRO and MSCA were granted to

me for returning to home country and continuing the research on applicability of laser technology for detection and structural analysis of amyloid protein fibrils. I'm also a principle investigator of Polish part in the project funded from EU joint programme – neurodegenerative disease research (JPND). The multinational project has six member states whereby the consortium focus is on the detection of early symptoms of neurodegeneration and protein oligomers. The Polish part of the project is financed by NCN with additional funds from EU JPcofuND 2.

I'm a laureate of the scholarship for outstanding young researcher from Ministry of Science and Higher Education. I was also the laureate of START programme of Foundation for Polish Science and the first recipient in the history of the of the Barbara Skarga scholarship for a young scholar whose research displays courageous breaking of interdisciplinary boundaries, opening new research perspectives and creating new values in science. I received also an award from Wroclaw Town President for outstanding research achievements.

The field of early diagnostics of neurodegenerative diseases, which I continuously work on for 8 years, is one of the scientific priorities of the European Union member states because of aging population problem in Europe. My research record, documented with the list of publications and awarded grants from national and international institutions, shows my commitment to the field of the brain diseases. I collaborate and develop the research schemes with leading national and international laboratories focused on laser optics for applications in research on amyloid fibrils structure and diagnostics of neurodegeneration diseases.

In respect to my scientific achievements and research career track I would like to apply to the Council of Excellence for considering my application for habilitation degree.

Cycle of scientific articles included in the habilitation cycle „Laser spectroscopy methods for detection of aggregated proteins and peptides doped with fluorescent markers" in an chronological order:

1. **P. Hanczyc**, M. Procyk, C. Radzewicz, P. Fita*, *Two-Photon Excited Lasing of Coumarine 307 for Lysozyme Amyloid Fibrils Detection,* Journal of Biophotonics (**2019**), 12 (9), e201900052;

2. **P. Hanczyc***, A. Justyniarski, J. Kim, A. Mikhalovsky, M. Ivanova, *Surface Patterns of Insulin Fibrils Revealed by Time-Resolved Spectroscopy Measurements of Fluorescent Probes,* J. Luminescence (**2018)**, 201 (9), 31-37;

3. **P. Hanczyc***, A. Mikhailovsky, D. Boyer, M. R. Sawaya, A. Heeger, D. Eisenberg*, *Ultrafast Time-Resolved Studies on Fluorescein for Recognition Strands Architecture in Amyloid Fibrils,* J. Phys. Chem. B (**2018**), 122 (1), 8–18;

4. **P. Hanczyc***, L. Sznitko, *Laser-Induced Population Inversion in Rhodamine 6G for Lysozyme Oligomer Detection*, Biochemistry, (**2017**), 56 (22), 2762-2765;

5. **P. Hanczyc**, L. Sznitko, S. Zhong, A. Heeger*, *Stimulated emission from rhodamine 6G aggregates self-assembled on amyloid protein fibrils*, ACS Photonics (**2015**), 2 (12), 1755-1762;

6. **P. Hanczyc***, A. Justyniarski, D. A. Gedefaw, M. R. Andersson, M. Samoc and C. Müller, *Two-photon absorption of polyfluorene aggregates stabilized by insulin amyloid fibrils*, RSC Advances (**2015**), 5, 49363-49368;

7. L. Sznitko*, **P. Hanczyc***, J. Mysliwiec, M. Samoc, *Low-threshold stimulated emission from lysozyme amyloid fibrils doped with a blue laser dye*, Applied Physics Letters (**2015**), 106, 023702;

8. **P. Hanczyc***, *Binuclear Ruthenium(II) Complexes For Amyloid Fibrils Recognition*, Chemical Physics **(2014)**, 445 (12), 1-4;

9. **P. Hanczyc**, M. Samoc, B. Norden*, *Multiphoton absorption in Amyloid Protein Fibres*, Nature Photonics (**2013**), 7 (12), 969-972;