**Review on the scientific achievement by Dr Pawel Ferdek for the attribution of the post-doctoral degree of Doctor habilitated in the field of natural sciences in the discipline of biological sciences**.

Dr Pawel Ferdek is presently a member of the Department of Cell Biology of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University in Krakow. To reach this faculty position the studies and early research career that Dr Pawel Ferdek has followed could be mainly illustrated by:

Faculty studies leading to the Master degree of Science and Master degree of Research marked by stays achieved through international collaboration with USA and UK where the research stay was done (University of Liverpool). This was continued by the 4-year PhD performed in the school of Biosciences of the Cardiff University (2008-2012). The topic of interest that characterizes Dr Pawel Ferdek's work started there and was devoted to understanding the mechanisms of pancreas pathologies, as illustrated by the title of his PhD thesis: *The role of Bcl-2 family proteins and calmodulin in calcium signalling in pancreatic acinar cells.*

The research was funded by a prestigious Welcome Grant for which Mgr Pawel Ferdek was the PI.

The post-doctoral research stays (6 years) were performed in the same University and same school and group, funded by two grants, one of which the candidate was the PI on a very important and up to date theme of organoid development.

Besides the fact that the post doc stays were done in the same group, the international cooperation and links requirements are quite fulfilled since Dr Pawel Ferdek started an international research carreer by an early internship in Prof. C. Hedrick's team, University of Virginia, Charlottesville, VA, USA while he was a master student in the Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow.

It has to be remarked that the scientific work has been carried out at several academic institutions other than the Jagiellonian University (Alma Mater) both before and after the PhD.

The cooperative programs also involve institutions in Europe: University of Padua, International Centre for Genetic Engineering and Biotechnology Trieste, Italy, Medical University of Vienna, Karolinska Institutet, Stockholm, Sweden; University of Groningen, Groningen, Netherlands; Charles University in Prague, Prague, Czech Republic; Bogomoletz Institute of Physiology Kiev, and the National University of Lviv Lviv, Ukraine, which all gave the production of scientific publications (7). The special long term cooperation with the UK concerns the Cardiff University and the Universities of Liverpool, Oxford, Bath, Strathclyde but also GlaxoSmithKline Research Centre (total 12 publications).

The cooperation with USA involved the University of Virginia, the University of California in Los Angeles and in Davis, Beckman Research Institute of City of Hope Duarte, CA (5 publications).

The worldwide cooperation occurred with Japan (the RikenBrain Science institute leading to 2 publications) and with the Chinese team in West China Hospital Sichuan University, Chengdu (3 publications), and Department of Physiology Medical College Jinan University Guangzhou (2 publications).

Of course, inside Poland the collaborative activity is present as well, starting from the Faculty of Biochemistry, Biophysics and Biotechnology Jagiellonian University Kraków, Poland (7 publications); Malopolska Centre of Biotechnology, Jagiellonian University (3 publications); Faculty of Pharmacy Jagiellonian University Medical College (1 publication), to the University of Łódź, Łódź, Poland (1 publication).

Later on, during his post-doctoral work, then as a research fellow, he could establish collaborative research programs between the Cardiff and Xiamen Universities, then set it between the Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow and Sichuan University, Chengdu, China, with the production of three joint publications although the above described stays are quite short.

As an assistant professor for research, since 2018, the candidate has and is, participating in 7 grants either as a co-applicant for one NCBiR grant but as a PI/grant holder for 4 grants: 2 HOMING grants are ended but the 2 NCN are ongoing (SONATA BIS ends in 2028). Moreover, as project supervisor for 2 grants the candidate has the possibility to be directing the research done by young scientists.

Indeed, the habilitation degree is by definition "habilitation to lead research", it means that the candidate must be able to teach young scientists to be good scientists, able to conduct research by themselves when they reach the doctorate degree. In Dr Pawel Ferdek's instance the condition is fulfilled by his activity in supervising MSc students (4 to date) as well as being the co-supervisor for 2 ongoing PhDs (to be defended in 2024 and 2025).

As a member of the university, the didactic capacity is shown through the teaching of university courses. Dr Pawel Ferdek has been indeed teaching practicals at the university of Liverpool first, then lecturing at the Jagiellonian University (from 2021 to date) on Cell Biology and Cytochemistry.

Similarly, the evaluation of the research achievement by students during their formation is a necessary step. Dr Pawel Ferdek, did it since his entry as a research assistant by reviewing the BSc dissertations in Biotechnology (2) and in Biochemistry (1), as well as the MSc dissertations in Biotechnology (2).

The organizational activity is necessary and should be documented through the organization of symposia or co-organization of congresses. For the time being Dr Pawel Ferdek has started such action through his action as a member of the organizing committee of the Krakow Interdisciplinary Science Seminar (KISS) 2019 – a meeting for FNP grant holders as well as in 2020, being a Member of the organizing committee of the Krakow Interdisciplinary Science Seminar (KISS) at MCB – a meeting for FNP grant holders, at BBiB.

In terms of editorial activity, Dr Pawel Ferdek acted as an Editorial Board Fellow at The Journal of Physiology (ISSN: 1469-7793, IF2021=6.228) and Guest Editor for Frontiers in Physiology (ISSN: 1664-042X, IF2021=4.775), as well as, presently being a Reviewing Editor at The Journal of Physiology (ISSN: 1469-7793, IF2021=6.228).

This activity has been recognized and awarded since 2016 by the Joint Cardiff/Xiamen Grant, to visit the Chinese university of Xiamen and for the excellence in academic achievement by the Academia Europae Burgen Scholar Award (2016). The quality of the research was clearly appreciated by the acceptation to participate in the Gordon conferences (twice) with a travel grant by the Physiological Society. More recently, 2020 The Polish Intelligent Development Award in the Scientist of the Future category was awarded together with a Scholarship for Outstanding Young Scientists from the Ministry of Science and Higher Education (Poland) that were obtained, showing a discipline recognition.

Considering the scientific achievement that constitutes the matter of the habilitation application, Dr Pawel Ferdek has put together 7 articles among which 5 original papers and 2 reviews in which he acts as corresponding author and he appears either as first author (2013-2017) then (2019,2022) as senior author. As the impact factor of those publications ranges 4.0 to 12.78, it means that the level of the candidate's research is undoubtedly high.

The description of the selected works is developed along 12 pages didactically and synthetically. The introduction to the theme clearly links the publications and the progression of the research appears logically to point to the Ca2+ signaling and cells death in the pathophysiology of distinct cells in the pancreas

Title:

*Cellular mechanisms underlying the diseases of the exocrine pancreas – the role of Ca2+ signaling and Bcl-2 family proteins in pancreatic acinar and stellate cells*

The first noticeable work, performed during the PhD research and published in 2013 in PNAS, IF=12,779 was achieved by the team among which the candidate did his PhD, with a substantial experimental contribution about the mechanism of action of the Ca2+ in pancreatic acinar cells.

Using the first drug inhibitor of SOCE, Ca2+ influx after depletion of the ER Ca2+ store reduced in PACs and corresponds with the pathophysiological [Ca2+]i elevation. It shows that inhibition of excessive Ca2+ influx through the SOCE mechanism effective against [Ca2+]i overload, necrosis, does not affect physiological Ca2+ signals in PACs and that CRAC channel inhibition is potentially a strategy against AP.

This largely cited study, and remarked by Cardiff University "Scientists edge closer towards first pancreatitis treatment", was followed by confirmation of results in mouse pancreatitis (Wen et al., 2015), with a strong impact on pancreatic research field (Waldron et al., 2019).

In 2016 the work: "Bile acids induce necrosis in pancreatic stellate cells dependent on calcium entry and sodium-driven bile uptake" with P. Ferdek as first author was published in J Physiol (London); IF = 6,228 was published.

This study proves the distinct activity of stellate and acinar cells despite the very close proximity, with new insights into the mechanisms behind the bile acid-induced pancreatic pathology, PACs and PSCs have clearly different sensitivities to bile acids, and distinct bile acid uptake (Na+ -dependent vs Na+ -independent). In both cell types, bile acids induce Ca2+ overload followed by necrosis.

The impact of this study was proven by the recognition of the scientific community through presentation at Gordon Research Conference (GRC) on Calcium Signalling, 2015 and a highlight (2016) titled: Bile as a key etiological factor of acute but not chronic pancreatitis: a possible theory revealed (Hegyi, 2016).

The further elucidation of the mechanisms was published in Cell death and Disease (2017): " BH3 mimetic-elicited Ca2+ signals in pancreatic acinar cells are dependent on Bax and can be reduced by Ca2+ -like peptides" by Dr Pawel Ferdek as first author showing that BH3 mimetics are toxic for PACs but also shows the molecular mechanism and role of Bax in intracellular Ca2+, and passive Ca2+ leak from the ERS.

It was the subject of a Research Highlight in The FEBS Journal (2017) and was selected as oral communication at the Gordon Research Seminar (GRS) on Calcium Signaling.

To deepen the possible applicability of the elucidation of the mechanism by which Ca2+ signaling is relevant for toxicity in PACs, occurred the 4$^{th}$ publication (2019) : "ABT-199 (Venetoclax), a BH3-mimetic Bcl-2 inhibitor, does not cause Ca2+ signaling dysregulation or toxicity in pancreatic acinar cells" in Br J Pharmacol (IF =9,473) with Dr Pawel Ferdek as senior author. In this paper the authors show the potential of such BH3 mimetic as being relatively safe for the pancreas when used for the treatment of patients with leukaemia.

This appears to be a quite powerful and new anti-leukemic agent safe for PACs with no significant pancreatotoxicity. This international collaboration publication (Belgium, Italy and UK), is the start of achievements where Dr P. Ferdek is senior author and is pursued in the 5th original research article proposed for the habilitation: "Activation of pancreatic stellate cells attenuates intracellular Ca2+ signals due to downregulation of TRPA1 and protects against cell death induced by alcohol metabolites" published in Cell Death Dis. 2022. Mainly showing for the first time that alcohol metabolites act not only on PACs but also on PSCs and inducing substantial [Ca2+]i overload and cell death, the paper also demonstrates that activated PSCs become resistant to alcohol metabolites as they are less prone to [Ca2+]i overload.

This paper is of potential high impact for pancreatic diseases, revising the dogmas on alcoholic pancreatitis. TRPA1 role in other tissue fibroblasts, is pointed in cell death regulation and could be a universal regulator for tissue (myo)fibroblasts.

Consequently, the work at this point appears indeed, sufficiently advanced and build a group of findings that are advancing considerably the knowledge on the pancreas biology.

This is confirmed by the updates brought by 2 reviews, one from 2017, making the state of the art and describing the important information on PSCs. Published in Pflugers Arch with Dr P. Ferdek as first author and IF=4.219, this paper is well cited, bringing large, useful and even necessary documentation on the topic.

The second review proposed here is: "When healing turns into killing - the pathophysiology of pancreatic and hepatic fibrosis", Dr Pawel Ferdek PE is first author and is published in J Physiol (London) 2022 with IF = 6.228. This is an important review since it gives the summing up of the older *vs* recent literature on pancreatic and liver fibrosis. It makes the link to general pancreatic/hepatic diseases from perspective of the cell physiology.

Considering the main achievements in the research described by Dr Pawel Ferdek who insists on the new insights on the exocrine pancreas brought in this work, namely : • Inhibition of store-operated Ca2+ entry could be a potential tool in the therapy against acute pancreatitis (Gerasimenko et al., 2013). • the distinct effects of bile acids on PACs and PSCs with PSCs being important targets of bile acid toxicity (Ferdek et al., 2016). • PSCs are expressing the NTCP, the main uptake pathway for cholate and taurocholate (Ferdek et al., 2016). • the implication of Bax in the passive Ca2+ leak from the ER in PACs (Ferdek et al., 2017). • BH3 mimetics on Ca2+ homeostasis is Bax dependent (Ferdek et al., 2017). •new-generation BH3 mimetic ABT-199 may be safe for the pancreas in the treatment of leukaemia (Jakubowska et al., 2019). • alcohol metabolites act on both PACs and PSCs inducing [Ca2+]i overload and cell death (Kusiak et al., 2022). • significantly higher resistance to alcohol of activated PSCs through downregulation of TRPA1 (Kusiak et al., 2022) and important 2 reviews on PSCs physiology and pancreatic / hepatic fibrosis, open perspective for new of research avenues in pancreatic (patho)physiology which is the criterion of a strong and innovative research.

Considering that the proposed habilitation group of articles are making a homogenous, logical and nicely progressing advancement of findings bringing strong new information on the pathology of the exocrine pancreatic cells, this reviewer appreciates the harmonious description of the work as well as the important research contribution made by Dr Pawel Ferdek.

It appears that Dr Pawel Ferdek, did part of his studies in the UK (doctorate and Post Doctorate). Although it is not recommended to perform such key steps of a carreer in the same place, it is also

appreciated that the candidate returned to Poland afterwards. Dr Pawel Ferdek could indeed, continue to collaborate internationally and become an important participant of the Department of Cell Biology, in the Faculty of Biochemistry, Biophysics and Biotechnology, of the Jagiellonian University in Krakow.

Dr Pawel Ferdek, along the carreer has also proven his didactic capacities as well as his ability to lead grants, but also his ability to lead the research of MSc students as well as doctoral students.

This reviewer strongly recommends that Dr Pawel Ferdek will receive the conferment of the post-doctoral degree of doctor habilitated in the field of natural sciences in the discipline of biological sciences.