ADAM MICKIEWICZ UNIVERSITY IN POZNAŃ
Faculty of Biology
Institute of Molecular Biology and Biotechnology

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Referee report concerning the **Habilitation thesis of Monikaben Padariya, Ph.D.** entitled as: ***"Developing pharmacophore concepts exploring structural basis of protein networks involved in cancer or immune response".*** International Centre for Cancer Vaccine Science, University of Gdańsk, Poland.

Education and professional achievements

Dr Monikaben Padariya has been awarded a MSc and engineering degree in Biotechnology/Bioinformatics, in 2012 at the Faculty of Chemistry, Wrocław University of Science and Technology, Poland. She presented a Thesis entitled „*Computer-aided design of organophosphorus inhibitors of urease*" (supervised by Profs. Wacław A. Sokalski and Łukasz Berlicki). She received an additional training (June-September 2015) at the Indian Institute of Technology in New Delhi at the Supercomputing Facility for Bioinformatics and Computation Biology (Led by Prof. B. Jayaram) working on a project related to "*Designing new compounds based on dibenzopyrrole structure to improve its efficiency as telomerase inhibitors applying fragment based approaches*". She has later conferred the PhD degree (dr inż.) in 2018, (supervisor: Prof. Maciej Bagiński), with the research results summarized in the Thesis entitled "*Structural and dynamic insights on the EmrE protein in apo-form and with TPP+ related substrates*". The research towards PhD degree has been funded by Polish National Agency for Academic Exchange (NAWA). Dr Padariya has been employed as a Scientific researcher at the Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology (yrs 2018-2019). Since 2019, Dr Padariya is working as an adjunct at the International Centre for Cancer Vaccine Science, University of Gdańsk, Poland.

Scientific achievement

The candidate has presented the scientific achievement related to the development of various pipelines serving individualized therapies. Such approaches combine gathering large data utilizing multi-omics approaches, i.e. from proteomics, interactomics and structural analysis, as well as big data analysis, and molecular modeling among others. Dr Padariya has developed pharmacophore models of various molecular systems participating in dedicated protein-protein protein-DNA or protein-RNA interactions, providing insight on allosteric sites. Such pipelines are useful in the analysis of disease-related phenomena, especially concerning the highly genetically variable and molecularly complex, multifaceted disorders, like cancer and in cases when the archived material is scarce, and hard to be assessed by the established research or clinical

methods (i.e. SARS-CoV2 infections). The dissertation is based on **seven peer-reviewed original articles and one review**, ranging from a middle to high rank, mostly specialized, open access journals (years 2021-2022). The overall impact factor (IF), Ministry of Science and Higher Education (MNiSW) journal scoring values, and the number of citations were adequately provided by the candidate, and included those from Web of Science and Google Scholar (both accessed on August 22, 2023), according to the given guidelines (in the Scientometric information, chapter IV). **In all eight articles, the candidate was the first author, and in five of them served as the (co-)corresponding author**. The summarized 5-year IF of publications belonging to the scientific achievement amounts to **56.524** (**MEiN = 910 points**), which represents a **very good level of scientific achievement**. These articles were cited **32** times (WoS) times (accessed on 19/05/2023, WoS), and **40/48** (Scopus and Google scholar respectively), demonstrating rather a decent degree of international recognition, which is important when topics with advanced technical content explaining the important biological phenomena are being evaluated. It is **very likely that the number of citations will expand** judging the fact that they were **published only recently**.

The following articles have been presented by the candidate:

1. Padariya, M., Jooste, M. L., Hupp, T., Fåhraeus, R., Vojtesek, B., Vollrath, F., Kalathiya, U., & Karakostis, K. (2022). The Elephant Evolved p53 Isoforms that Escape MDM2-Mediated Repression and Cancer. Molecular biology and evolution, 39(7), msac149. https://doi.org/10.1093/molbev/msac149. **IF_{2020}=16.24**
2. Padariya, M., Kote, S., Mayordomo, M., Dapic, I., Alfaro, J., Hupp, T., Fahraeus, R., & Kalathiya, U. (2021). Structural determinants of peptide-dependent TAP1-TAP2 transit passage targeted by viral proteins and altered by cancer-associated mutations. Computational and structural biotechnology journal, 19, 5072–5091. https://doi.org/10.1016/j.csbj.2021.09.006. **IF_{2020}=7.27**
3. Padariya M, Kalathiya U, Mikac S, Dziubek K, Tovar Fernandez MC, Sroka E, Fahraeus R, Sznarkowska A. Viruses, cancer and non-self recognition. Open Biol. 2021 Mar;11(3):200348. https://doi.org/10.1098/rsob.200348. **IF_{2020}=6.41** (**review article**)
4. Padariya, M., Fahraeus, R., Hupp, T., & Kalathiya, U. (2021). Molecular Determinants and Specificity of mRNA with Alternatively-Spliced UPF1 Isoforms, Influenced by an Insertion in the 'Regulatory Loop'. International journal of molecular sciences, 22(23), 12744. https://doi.org/10.3390/ijms222312744. **IF_{2020}=5.92**
5. Padariya M, Kalathiya U. The Binding Specificity of PAB1 with Poly(A) mRNA, Regulated by Its Structural Folding. Biomedicines. 2022 Nov 19;10(11):2981. https://doi.org/10.3390/biomedicines10112981. **IF_{2020}=4.757**
6. Padariya, M., Daniels, A., Tait-Burkard, C., Hupp, T., & Kalathiya, U. (2022) Self-derived peptides from the SARS-CoV-2 spike glycoprotein disrupting shaping

and stability of the homotrimer unit, Biomedicine & Pharmacotherapy, 151, 113190. https://doi.org/10.1016/j.biopha.2022.113190. **IF$_{2021}$=7.419**

7. Padariya, M., Sznarkowska, A., Kote, S., Gómez-Herranz, M., Mikac, S., Pilch, M., Alfaro, J., Fahraeus, R., Hupp, T., & Kalathiya, U. (2021). Functional Interfaces, Biological Pathways, and Regulations of Interferon-Related DNA Damage Resistance Signature (IRDS) Genes. Biomolecules, 11(5), 622. https://doi.org/10.3390/biom11050622. **IF$_{2020}$=4.88**

8. Padariya, M., Baginski, M., Babak, M., & Kalathiya, U. (2022). Organic solvents aggregating and shaping structural folding of protein, a case study of the protease enzyme. Biophysical chemistry, 291, 106909. https://doi.org/10.1016/j.bpc.2022.106909. **IF$_{2021}$=3.628**

In the first article of the achievement, Dr Padariya has dealt with one of the most studied genes/proteins, *TP53*, which plays pivotal roles in multiple cancers, neurodegenerative and developmental processes, tissue inflammation and regeneration, cellular senescence, aging as well as stress-induced mechanisms. The elephants have naturally evolved several p53 isoforms of variable lengths and sequences. In this work, Dr Padariya has employed bioinformatic modeling and identified the homology of the BOX-I motifs of p53 isoforms from the elephant *L. Africana,* and calculated their docking capacities to MDM2, the main p53 regulator. These results have implications on downstream cell signaling mechanisms, cellular senescence to drive lifespan and body mass, constituting potentially critical therapeutic or diagnostic targets. These interesting results have been highlighted in popular science article (Dlaczego-slonie-nie-choruja-na-raka-badania-naukowcow-z-Uniwersytetu-Gdanskiego).

In the second article, Dr Padariya embarked on deciphering of how the cancer-derived mutations in TAP1-TAP2 or viral factors targeting the peptide loading complex, PLC, interfere with peptide transport. The obtained results provided a model for how viruses and cancer-associated mutations targeting TAP interfaces can affect MHC-I antigen presentation, and how the IFN-c pathway alters MHC-I antigen presentation via the kinetics of peptide transport.

The third review article of the achievement, Dr Padariya focused on virus–host interactions, and particularly on the immune system. Several aspects have been covered including routes of virus–host coevolution, the self- identity of the viruses, the role of the major histocompatibility class I pathway and the viral interference, aspects of viral and cancer immune evasion, the impact of viral infections on immune checkpoints, description of the source of antigenic peptides emerging from immune evasion to cell cycle control, and finally, the non-protein-mediated control of the host cell. Importantly, viral immune evasion strategies are intertwined with multiple cellular pathways. Immune evasion is targeted by cancer cells, while interfaces amid viral factors and components of the MHC class I PLC serve are sites of multiple cancer mutations.

The fourth article dealt with the process of the nonsense-mediated mRNA decay (NMD), which eliminates the defective mRNAs with premature termination codons (PTC), one of the processes that regulate the mRNA surveillance quality control pathways. In particular, the UP-frameshift 1 (UPF1), the master regulator of the NMD process, that has two alternatively-spliced isoforms with different mRNA motifs, was the focus of the article. The authors traced changes in local dynamics by inserting cancer-derived mutations in the UPF1 mRNA binding pocket. From the gathered data it has been hypothesized that the increased affinity between UPF1-mRNA components shall aid in enhancing the RNA-dependent ATPase/helicase activity of the UPF1 protein. Novel perspectives from identified mRNA UPF1 binding pairs may further reveal the selectivity of respective partners, as well as scrutinize NMD-associated dynamics.

The fifth article of the achievement focused on poly(A)-binding protein cytoplasmic 1 (PAB1 or PABPC1), associated with the long poly(A) mRNA tails inducing its stability. PABPC1 protein has been proposed to act as an antagonist of the NMD factors when targeting mRNA. Deletion of either PAB1 or UPF1 (NMD) significantly increases the production of novel peptide read-through and may result in the increased production of mutant peptides that could trigger the immune response in cancer. The dynamics of the PABPC1 protein, along with tracing its mRNA binding specificity was investigated. Changes in RRM3–4 domains (RNA recognition motifs) initiated a folded structure that induced different conformational folds. The majority of the high-frequency cancer mutations in PABPC1 reside within the RRM4 domain, and amino acids engaging in high-frequency interactions with poly(A) mRNA were found to be preserved in multiple cancers. These data can be used to modulate the activity of *PABPC1*, resulting in generation of mutant peptides or neoantigens in cancer.

The sixth article of the achievement of Dr Padariya focused on the structural spike (S) glycoprotein from the SARS-CoV-2 β-coronavirus. Importantly, to support the ongoing novel vaccine design and development strategies, the structure-based design approach to develop self-derived S peptides was proposed. Critical regions of the S protein may act as blockers or stabilizers of homotrimer unit. Self-derived peptides from the S protein were thus screened against the second or third monomer of the homotrimer, to elucidate whether they can stabilize / block the trimer unit. Besides, point mutations emerging from different SARS-CoV-2 virus variants with respect to change in the protein-peptide binding affinity were evaluated. Significant changes in the region where protein-peptide forms a high number of H-bonds interactions are linked with frequent mutations. These findings can aid in designing self-derived peptides replacing a single S protein monomer from the trimer, thereby blocking or inducing stability of the homotrimer.

The seventh article dealt with Interferon (IFN)-related DNA damage resistant signature (IRDS) genes. They form a subgroup of interferon-stimulated genes (ISGs) upregulated in different cancer types, in turn evoking resistance to

DNA damaging chemotherapy and radiotherapy. The performed pathway enrichment analyses revealed an association of IRDS genes with various biological pathways, connected to the 'interferon viral regulation', and excluding the 'negative regulation of endopeptidase activity'. Another highly populated biological pathways with the IRDS genes were also identified, including *IFIT1/3*, *IFITM1*, *IRF7*, *ISG15*, *MX1/2* and *OAS1/3/L*. The genes *OAS1*, *OAS3*, and *IFIH1* defined their pharmacophore with dsRNA, in a similar manner as the STAT1 and IRF7 proteins. The Lys residue was shared in *OAS1*, *EIF2AK2* and *IFIH1*, associating with the P- groups of the ATP molecule, while *MX1* and *HSD17B1* classified a conserved active site with the GDP and NADP+ moieties. These findings warrant novel approaches to target the identified functional sites in IRDS, and possibly other gene families.

The eighth article of the cycle focused on seemingly different topic, exploring how aqueous solutions containing different organic solvents / deep eutectic solvents (DESs) may influence the protease enzyme's activity, structural, and thermal stabilities. For this purpose, the retroviral aspartic protease enzyme responsible for the cleavage of the polypeptide precursors into mature viral components was chosen. In molecular dynamic simulations, the complex of the protease enzyme with *Darunavir* (one of the most common HIV-1 protease inhibitors) was found highly stable in urea aqueous solution as compared to ethylene glycol or glycerol solvents. *Darunavir* ligand induced interactions amongst monomers of protease in different aqueous solutions. These findings indicate that self-aggregation within a particular type of organic solvent has a significant effect over the folding of the HIV-1 protease and its binding to *Darunavir*. In conclusion, organic solvents can have a significant influence on nonpeptidic inhibitor activities towards the proteases, suggestive for a direction of further *in vitro* and *in vivo* experiments.

In summary, the presented articles constituting a pivotal part of the achievement represent a solid, concise methodological ground for the further development of bioinformatic methods dealing with pharmacophore models in clinical setup. In reviewer's opinion, the candidate has successfully gathered, analyzed and developed the methodologies and tools, which can be utilized by other researchers in the structural bioinformatics field. Thus, **this part of the dissertation very clearly fulfils the criteria** set for obtaining the habilitation degree.

Evaluation of other scientific achievements

Dr Monikaben Padariya has published in total **33** articles, in **17 of which she was the first author**. During the course of a doctoral degree she has published 16 articles. Following conferment of her PhD degree in 2018, Dr

Padariya has successfully published another 17 articles, of which eight is included as part of the scientific achievement. Research of Dr Padariya has been cited in total **212 times** (access on 22/08/2023; WoS), while her Hirsch index (**HI**) from the same database **equals 9**. Recent Google scholar parameters, also provided by the candidate, list in total **313 citations** and **HI of 10** (access on 22/08/2023). Dr Padariya has also contributed to one patent application in 2022 (PZ/8885/RW/PCT), concerning the *Inhibitors of interactions between TRF1-TIN2 or TRF2-TIN2 telomeric proteins for use in anticancer therapy*). **In summary, these parameters are quite decent concerning the time of obtaining the PhD thesis (2018) and a period of maternity leave,** although the overall number of citations is somewhat **moderate**.

Concerning the management of international and national research projects and participation in such projects as a project executor, Dr Padariya has listed **six projects**, which included the University of Gdańsk Programme of Small Grants (second edition, 2022; https://iccvs.ug.edu.pl/news/eight-ugrants-go-to-iccvs/) as a **PI**, SONATINA NCN (**2020-23**; 2020/36/C/NZ2/00108, *Specificity in detection of PTCs in mRNA by NMD and its network, insights from cancer perspective and cross-linking (XL-MS)*; projekty.ncn.gov.pl/475917) as **co-PI**, OPUS NCN (**2021-25**; 2020/39/B/NZ7/02677, *The impact of UPF1 ATP mimetics on the mutant immunopeptidome;* projekty.ncn.gov.pl/502673) as **co-PI**, The National Center for Research and Development, Poland (TARGETELLO, **2017-18**, *New compounds with anticancer activity that disrupt telomere functions*) as **co-PI**, SONATA NCN (**2022-25**, 2021/43/D/NZ1/02059, *Characteristics of the function and regulation of isoform 2 of the Nrf2 transcription factor;* projekty.ncn.gov.pl/534074) as a co-PI, and OPUS NCN (**2015-16**, 2014/13/B/NZ7/02207, *New inhibitors of catalytic subunit of telomerase;* projekty.ncn.gov.pl/260664) as a co-PI.

Judging from the published articles and gathered experience of the candidate in collecting the necessary funding **offers a very promising perspective for successful conduct of high-level research and gathering an adequate funding as a PI in the future**.

It is worth emphasizing that during her doctoral studies, Dr. Padaryia completed several months of internship (mobility) in the group of Prof. B. Jayaram at the Supercomputing Facility for Bioinformatics and Computation Biology (SCFBio, Indian Institute of Technology, in New Delhi, India: www.scfbio-iitd.res.in). Moreover, she was awarded for three consecutive years, the title of the best doctoral student at the Gdańsk University of Technology and awarded twice (Young scientists award, in 2015 and 2017) by the Polish Academy of Sciences for "the best creative work published". The candidate also received a Ignacy Łukasiewicz PhD scholarship funded by the Polish National Agency for Academic Exchange (NAWA). In 2021, Dr Padariya received the 3rd degree award of Rector from Uni. of Gdańsk for achievements in her scientific work.

Dr Padariya, has listed eight other achievements (parameter International and National awards for scientific or artistic achievement), of which majority relates to her successful PhD thesis work, with the last one awarded in 2018. Concerning the oral and poster presentations (3 in total) at the national and international level, **only eight** were enumerated, with the last one given in 2023 at the SBME conference in Ferrara-Italy. Concerning the participation in European programs and other international and national programs, Dr Padariya has contributed to reviewing of several peer-reviewed articles although the exact number and the specific journals are not listed, except those in *Scientific Rep*, *IEEEE/ACM Transactions Comp Biol Bioinf* and *J Biomol Struct Dyn* (1 review each listed).

Lack of considerable national/international mobility following the conferment of a PhD degree (in 2018) has been rationally explained by the candidate due to the COVID pandemia and maternity leave. It can also be counterbalanced by the established international collaborations, i.e. with Dr K. Karakostis - Universitat Autònoma Barcelona, Spain; Prof. F. Vollrath - University of Oxford, UK; Dr Ch. Tail-Burkard - University of Edinburgh, UK; Prof. B. Vojtesek, RECAMO, Masaryk Memorial cancer Institute, České Budějovice, Czechia; Dr M. Babak - University of South Bohemia, Czechia; Dr S. Chakraborti - National Institute of Malaria Research, New Delhi, India.

Dr Padariya served as an Editor of the special issue “SARS-CoV2 Spike-Based Vaccines” in the *Vaccines* (MDPI) journal (https://www.mdpi.com/journal/vaccines/special_issues/MA3581UT01), in 2023.

Judging all above, this part of presented achievements demonstrates a **strong capability of a candidate in conducting high quality research, establishing the national/international collaborations**, although displays rather **moderate experience of the candidate at the international forum** as a speaker, organizer and a senior researcher.

Teaching achievements and in the field of the popularization of science or art

Since the beginning of the employment at the ICCVS, Gdańsk, Dr Padariya has been/is relatively sparsely involved in supervision of MSC/PhD students. Taken together, the candidate possess moderate level of teaching experience demonstrated by few educational activities at different levels She has been tutoring in the Computer science laboratories at the Department of Physical Chemistry , Faculty of Chemistry, Gdańsk University of Technology (2014-16; Environmental Protection course in English). The candidate also serves as an auxiliary supervisor of the doctoral student work, currently being carried out at the ICCVS.

Final statement

Taking into account the overall achievements of Dr Monikaben Padariya, I believe that **she fulfils the formal criteria for obtaining the degree of habilitated doctor**, and further the requirements of the ACT Law on Higher Education and Science; Journal U. 2018, item 1668 of July 20, 2018. Having stated this, I also wish to pinpoint minor deficiencies, which were described in detail in the report given above.

Hereby, I submit to the High Scientific Council of the Biotechnology Division at the University of Gdańsk, an admission of Dr. Monikaben Padariya to further stages of the habilitation procedure.

Sincerely yours,
Maciej Maurycy Łałowski

Maciej Lalowski, PhD, DSc, visiting prof.
Department of Gene Expression
Institute of Molecular Biology and Biotechnology
Faculty of Biology,
Adam Mickiewicz University
Uniwersytetu Poznanskiego 6 St.
61-614 Poznań, Poland
Tel. (mobile) +48-797991432
E-mail: maciej.lalowski@amu.edu.pl
https://maclal1-polonezbis.web.amu.edu.pl/
https://ibmib.web.amu.edu.pl/groups/omics-diabetes-group/

Maciej M. Lalowski, Ph.D., D.Sc., Associate professor
Principal investigator
Medicum
Biochemistry/Developmental Biology
Meilahti Clinical Proteomics Core Facility
PO Box 63 (Haartmaninkatu 8), Room C214a
FI-00014 University of Helsinki
Finland
Tel. (office) +358-294125203
Tel. (mobile) +358-407790950
e-mail: maciej.lalowski@helsinki.fi

Signed by / Podpisano przez:

Maciej Maurycy Łałowski
Uniwersytet im. Adama Mickiewicza w Poznaniu

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