University of Gdańsk
International Centre for Cancer Vaccine Science

Developing pharmacophore concepts exploring structural basis of protein networks involved in cancer or immune response

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Summary of Professional Accomplishments

dr inż. Monikaben Padariya

The field of **Natural sciences**
The discipline of **Biotechnology**

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1. **Name:** Monikaben Padariya

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**

2018	Ph.D. (dr inż.) in chemical sciences in the field of Biotechnology, obtained at Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, Poland. My doctoral dissertation was entitled: Structural and dynamic insights on the EmrE protein in apo-form and with TPP^+ related substrates, supervisor: prof. dr. hab. inż. Maciej Bagiński.
2012	Master of Science and Engineering (mgr inż.) degree in Biotechnology / Bioinformatics, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland. My master's thesis is entitled: Computer-aided design of Organophosphorus inhibitors of Urease, supervisors: prof. dr hab. inż. Wacław Andrzej Sokalski and prof. dr hab. inż. Łukasz Berlicki.

3. **Information on employment in research institutes or faculties / departments or school of arts**

From 2019-present	Adiunkt / Postdoctoral researcher, at International Centre for Cancer Vaccine Science (ICCVS), University of Gdańsk, Poland.
2018-2019	Scientific Researcher, at Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, Poland.

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

My scientific accomplishment includes 8 scientific articles (6 research papers and 2 review publications), which are a collection of monothematic series demonstrating the pharmacophore models of diverse sets of proteins or enzymes involved in cancer or immune response. Pharmacophores were generated based on protein-protein or mRNA or DNA networks that provide insights on the active or allosteric sites. These works are published between 2019-2022 years describing basic science along with applied studies and are the outcome of collaboration with different academic research universities. The points are received for them according to article 219 paragraph 1 point 2 of the Act on Academic Degrees and Titles and on Degrees and Titles in Art. In those 8 publications representing my scientific achievements I am the first author in all articles, as well as corresponding author in 5 publications. These collective papers demonstrated as scientific achievements all together have a total Impact Factor (IF) of 56.524, and a total of MEiN (Ministerstwo Edukacji i Nauki, or (MNiSW) Ministry of Science and Higher Education) points awarded for the achievement is 910 (Ministry of Education and Science, Republic of Poland).

a) The title of the scientific achievement

Developing pharmacophore concepts exploring structural basis of protein networks involved in cancer or immune response.

b) The research publications belonging to the Scientific Achievement

4.1. 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; $MEiN_{2020}$ = 200**).

4.2. 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; $MEiN_{2021}$ = 100**).

4.3. Padariya, M., Kalathiya, U., Mikac, S., Dziubek, K., Tovar Fernandez, M. C., Sroka, E., Fahraeus, R., & Sznarkowska, A. (2021). Viruses, cancer and non-self recognition. Open biology, 11(3), 200348. https://doi.org/10.1098/rsob.200348; (**IF_{2020} 6.41; $MEiN_{2021}$ = 100**).

4.4. 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; $MEiN_{2021}$ = 140**).

4.5. Padariya, M., & Kalathiya, U. (2022). The binding specificity of pab1 with poly(A) mRNA, regulated by its structural folding. Biomedicines, 10(11), 2981. https://doi.org/10.3390/biomedicines10112981 (**IF_{2021} 4.757; $MEiN_{2021}$ = 100**).

4.6. 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; $MEiN_{2021}$ = 100**).

4.7. 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; $MEiN_{2021}$ = 100**).

4.8. 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; $MEiN_{2021}$ = 70**).

IF – impact factor from the year of publication of the work, and if the IF was not present for the specific year, the IF of the previous year was used. **MEiN** – journal scoring according to the

Ministry of Education and Science / Ministerstwo Edukacji i Nauk. Publications from previous **MEiN** scoring points were scored according to the new **MEiN** scores.

c) The discussion of the scientific objective of the above-mentioned work and the results achieved, together with a discussion of their application

Introduction

Aging related diseases including cancer have raised major health burdens worldwide, and therefore, new therapeutic drugs or vaccines that can influence patient prognosis are an immense need for better health care of the global community. Our immune defense system has strong efficiency to detect and eradicate distinct pathogens and malignant cells, maintaining or enhancing immune response can be vital for cancer therapeutics. Additionally, precision medicine or personalized medicine, improving targeted therapies for specific groups of cancer patients merge different scientific directions such as big data analytics, structural biology etc. required continuous development of new drug candidates. In this direction, my scientific activities include developing pharmacophore models of diverse sets of proteins or enzymes based on protein-protein or mRNA or DNA networks. Detailed understanding structural perspectives of different components involved in immune response or suppressing tumors is a crucial step to treat cancer and viral infection.

The p53 gene is a transcription factor whose primary function is to maintain cellular homeostasis in response to genotoxic stress signals and mutated or over-expressed in >30% of cancer types is one of the most common alterations in cancers. Normally, upon activation, p53 can cause cell cycle arrest, senescence, or apoptosis depending upon the condition and stress [1] and plays a vital role in chemotherapy and radiation therapy against different cancers. Given these important roles, it is obvious why so many human tumors require the loss of function of p53 to progress to a fully malignant phenotype. Considering activity of this gene across species, for e.g., the elephant's genetic set-up innately confers functional diversity to p53-dependant mechanisms contributing significantly to valuable protein fine tuning. This is in stark contrast to the human set-up where one gene with alternatively activated isoforms is expressed in response to a stress. The existence of multiple p53 forms in elephants, with potentially different activities, provides an ideal model for understanding how p53 activities are executed and regulated. Indeed, substitutions made by retrogene isoforms altering the p53 DBD (DNA binding domain) and its functions have been investigated and associated with longevity in species lifespan [2].

Important for an understanding of p53 diversity in elephants, Sulak et al. [3] investigated the roles of retrogene isoforms (TP53RTGs) forming a "pool of protected p53" thus acting as contributors to an enhanced sensitivity of elephant cells to DNA damage. The two models (the "guardian" or the "decoy" model) explored the potential roles of the TP53RTGs in facilitating the stabilization of the canonical p53, by either participating on the dimerization with canonical p53, or by acting as antagonists, competing for the mdm2 interaction [3]. We explored the elephants' apparently unique system further by focusing on the p53–mdm2 interaction. These interactions are crucial as p53 is primarily regulated by mdm2 and their interaction depends on the BOX I motif (residing in the TAD I) [4], which is intrinsically disordered in turn implying greater likelihood of evolutionary changes [5]. This suggests that the variations identified on the elephant BOX I sequences potentially modify the binding epitope of mdm2, and thus, alter the expression levels and activation of p53. Despite the large body size and long-life span,

elephants exhibit a high resistance to cancer as cancer mortality is estimated to be <5%, as compared with humans reaching up to 25%. Implementing my structural biology expertise, I investigated structural properties of elephant p53 isoforms to test the hypothesis that p53 isoforms induce distinct pools of p53 proteins with variations on the epitopes interacting with mdm2.

Many of the gene mutations found in genetic disorders, including cancer, result in premature termination codons (PTC) and the rapid degradation of their mRNAs by nonsense-mediated mRNA decay (NMD) [6]. Human genetic disorders are caused by diverse types of mutations including nonsense mutations, frameshift mutations, and mutations that cause alternative splicing events, resulting in PTC. It has been estimated that up to 30% of all mutations resulting in human genetic disorders result in PTCs [7]. In addition, many acquired mutations in cancer, including those that disable p53, result in PTCs [8]. Transcripts carrying a PTC are targeted for rapid degradation before they can be translated into protein through a multistep process termed nonsense-mediated mRNA decay. The molecular mechanism of NMD has not been fully delineated, though working models have been proposed. When the translation complex pauses at a PTC that is upstream of the multiprotein exon junction complex (EJC), eukaryotic release factors recruit the RNA helicase UPF1, a vital component of the NMD mechanism [9]. It is known that NMD inhibition can be achieved via other mechanisms [8] and determined that modest 80% depletion of UPF1 can suppress NMD activity without diminishing the proliferation or survival of cells.

UPF1, an RNA-dependent ATPase is the master regulator of NMD and its ability to selectively target PTC-containing mRNAs depends on its ATPase and helicase activities, as well as on the phosphorylation of its N- and C-terminal domains. Considering the influence of 'regulatory loop' in the UPF1 gene and its control over the catalytic and/or ATPase activity, I investigated interactions of both UPF1 isoforms in the presence or absence of the poly(U) mRNA, datasets retrieved from the molecular dynamics simulation (MDS) technique. Additionally, it has been identified that UPF1 can bind with different PTC-containing mRNAs efficiently, but the molecular details are still not clear. Therefore, I participated in investigating the interactions of UPF1 isoform_1 (having a longer 'regulatory loop') with PTC-containing mRNA transcript models. The significant changes in the UPF1 isoform_1 binding with the mRNA upon inserting the most frequently occurring cancer mutations were also elaborated. The PAB1 protein is suggested to be an antagonist of UPF1 towards its mRNA activity, and thus, the binding pattern of both these proteins with the mRNA becomes very crucial. However, the work by Chan et al. [10] has shown that deletion of either UPF1 or PAB1, induces the production of (approx. 6-8 fold increase) novel peptide read-through. Hence, UPF1 and PAB1 proteins were investigated in the presence or absence of poly(U) and poly(A) mRNAs, respectively. These novel perspectives from identified mRNA-protein binding pairs can contribute to understanding the selectivity of respective partners, as well as advancing NMD associated structural dynamics.

Deletion of either UPF1 or PAB1, induces the production of novel peptide read-through that could trigger the immune response or immune system, and these proteasomal degraded peptides are proposed to be transported by the TAP1-TAP2 transporters. The TAP transporters belong to the superfamily of ATP-binding cassette (ABC) transporters. These transported peptide antigens are presented on the major histocompatibility complex class I (MHC-I) molecules. The cancer-associated mutations and viral proteins may share a common trend towards TAP transporters, which is attenuation of their activity. Substantial effort has been made to understand the peptides that are translocated by the TAP1-TAP2 transporters.

Impaired TAP function results in reduced surface expression of MHC-I, as the empty MHC-I molecules are unable to present antigens to the immune system and is a common target for viral as well as cancer immune evasion [11]. Approximately, 15–20% of all human cancers worldwide are linked to viruses and this percentage could grow in the future. Several viruses have evolved factors that block the function of TAP [12], including herpesviruses such as Epstein-Barr virus, human cytomegalovirus, and herpes simplex virus type 1 that establish lifelong persistence in the host [13]. Association of these viruses with certain types of cancer or human malignancies has been demonstrated and knowledge about their targets, as well as functioning mechanisms can be useful in the development of future antiviral treatment strategies [14]. In concert with this, I investigated the TAP1-TAP2 transporters at the atomic level with a particular focus on how viruses inhibit TAP-mediated peptide translocation at a molecular level.

Additionally, among the SARS coronaviruses family, the spike (S) protein has been found in its homotrimer form, in which individual monomers play an important role in the functional structure folding [15]. Two conformations for S homotrimeric form have been identified, i.e., pre- and post-fusion [16]. The pre-fusion S protein is found comparatively more unstable, the receptor binding domains (RBD) alternate between an "up" (open) and "down" (closed) conformations [17]. Despite different approaches to target SARS-CoV-2 virus, there is a lack of a detailed understanding in the direction that would block the homotrimer formation of the SARS-CoV-2 S protein. Considering recent studies on the S protein fusion mechanisms, we sought to block the homotrimer formation or trapping stabilized pre-fusion conformation by self-derived S peptides or linear motifs. Since the homotrimer is the functional unit of the spike protein, disrupting this homotrimer formation could block the viral entry into the host cell. In this line, I proposed linear motifs that were found involved in the protein-protein intramolecular interaction between monomers of the S homotrimer. An optimized SARS-CoV-2 S protein structure [18] was used to identify interactions between monomers and designing self-derived linear peptide motifs. If successful, blocking the trimer functional unit could have several significant impacts on understanding the SARS-CoV-2; it can induce immune response, a critical component for vaccinology, and a blocked monomer or stabilized homotrimer can induce production of antibody repertoire different from the "open" conformation.

My research direction substantially includes **optimizing and developing pipelines** that could explore pharmacophore models based on the **structural basis of protein or enzyme networks involved in cancer or immune response**. The publication number 4.1., highlights the structural and functional aspects of different p53 isoforms retaining the mdm2 interaction, combining in silico modeling and in vitro assays. The structural basis of the p53-mdm2 interfaces can be used for the development of highly specific antibodies to target neoantigens and cancer mutations that are difficult to target in conventional ways. A set of cancer derived mutations and viral components docked with the peptide-loading complex, in particular, the MHC-I and TAP transporters are elaborated in publication number 4.2. and 4.3. Viral proteins predominantly destabilize the TAP1 protein, compared to that of the TAP2. The cytosolic "open-state" transport channel of the TAP proteins may intake peptides from cytosol-towards-ER and was targeted by viral proteins. **A majority of viral proteins interact with regions in the PLC which are highly mutated in cancers.** The publications number 4.4. and 4.5. include structural properties and selectivity of UPF1 and PAB1 proteins with the poly(U) and poly(A) mRNAs, respectively. Pharmacophore models were constructed tracing mRNA interactions that could have potential use to **screen small molecules blocking the activity of UPF1 protein or eventually the NMD pathway**. Linear peptide motifs identified when

involved in the protein-protein intramolecular interaction between monomers of the SARS-CoV-2 spike homotrimer sought to block homotrimer formation is presented in publication 4.6. In parallel, publication number 4.7. and 4.8, explains functional interfaces of IRDS genes and effects of deep eutectic solvents over the active site residues, respectively.

Detailed description of the publications included in the scientific achievement

(i) 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.

Objectives of the work

The p53 gene has evolved from an ancestral p53/p63/p73 gene, which gave rise to three p53-related genes, with distinct roles in mammals [19, 20]. The TP53 transcription factor is a tumor suppressor with key roles in oncogenesis. **The full-length p53 includes TAD I, which is required for the induction of p53 target genes and proapoptotic factors, leading to cell cycle arrest (G1) or apoptosis.** An exciting study discovered that elephants have 20 copies, that is, 40 alleles of the TP53 genes, compared with the typical number of one copy found (so far) in all other mammals [3]. Despite the large body size and long-life span, elephants exhibit a high resistance to cancer as cancer mortality is estimated to be <5%, as compared with humans reaching up to 25% [21]. The variability or truncation of the **elephant p53 isoformic sequences indicates an enhanced functional diversity,** and the formation of diverse pools of functional p53 proteins. This would increase distinct structural characteristics conferring specialized interacting interfaces for key functional proteins such as mdm2 and downstream activating proteins.

In normal conditions, the mdm2 E3 ubiquitin ligase binds the p53 protein via the BOX-I motif and catalyzes the poly-ubiquitination of p53, targeting it for degradation via the 26S proteasomal pathway [5]. This suggests that the variations identified on the elephant BOX I sequences potentially modify the binding epitope of mdm2, and thus, alter the expression levels and activation of p53. Investigating the molecular evolution of the **p53–mdm2 system, we explored the structural and functional aspects of p53 isoforms retaining the mdm2 interaction**, combining in silico modeling and in vitro assays. In collaboration with dr hab inż. Umesh Kalathiya (from University of Gdansk, Gdansk), **I modeled initial structures of the BOX-I motifs of the elephant p53 isoforms in order to test the hypothesis that p53 isoforms induce distinct pools of p53** proteins with variations on the epitopes interacting with mdm2. This has significant implications for functional diversity and the integration of diverse signaling outcomes. In addition, considering the human mdm2 structure as the template, **I implemented the homology modeling approach and constructed the elephant mdm2 protein structure.** A specific set of elephants **p53 isoforms were docked over the mdm2 E3 Ubiquitin Ligase by me,** to identify the binding affinity and conformation pattern of p53 gene (elephant L. african). Along with comparing our findings with previous models, we discussed potential translational insights derived from the molecular evolution (interspecies) of the p53–mdm2 axis and the evolved structural modules. Additionally, we used in vitro synthesized peptides and recombinant versions of human mdm2, to experimentally confirm the interactions in vitro, employing sandwich ELISA (Figure 1).

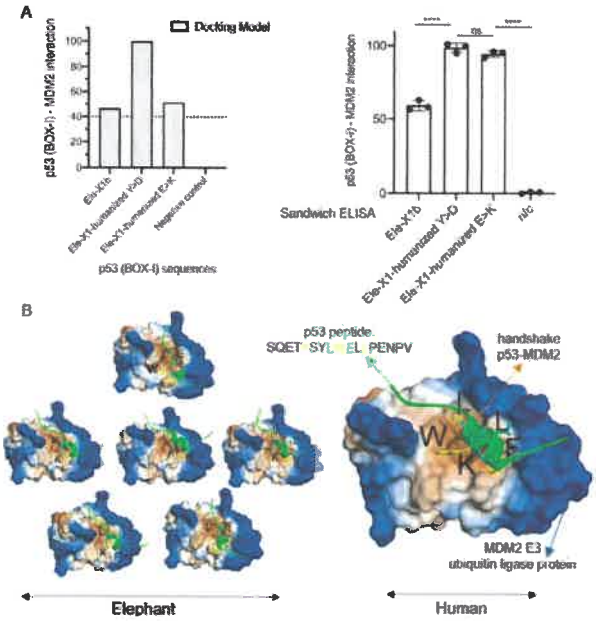

Figure 1. The mdm2–p53 N′ terminal (BOX-I) interaction, with calculated GBVI/WSA dG (–kcal/mol) docking values and normalized sandwich ELISA readings. **(A)** Docking model of the interaction of MDM2 with mutated Type A elephant p53: humanized Y>D or E> K. The humanized elephant p53 sequences exhibit an increased docking capacity, compared with the wt elephant p53 X1. The binding affinity values were normalized, setting the highest value at 100. **(B)** Illustration of the mdm2–p53 peptides docking models, explaining the association of each p53 peptide to mdm2, in humans and elephants. The hydrophobicity is visualized in blue for hydrophilic and brown for hydrophobic.

Description of results

Preventing the capacity of p53 isoforms to bind mdm2, directly promotes the stabilization, activation, and downstream signaling functions of p53. The p53–mdm2 interactions not only serve to control p53 expression but also as ligands to control their respective conformations and functions. We introduced the p53 isoforms from elephants as an interesting model system for structural studies of the p53–mdm2 interplay (Figure 1). Retrogene 12 along with retrogenes 14, 15, 16, and 18, form the group **"Type F," which shows poor binding to mdm2 strongly indicating that these putative proteins escape mdm2-dependent regulation.** In addition, our in silico analysis and the experimental ELISA findings both show that all the peptides carrying the **W>G variation exhibit decreased binding activity with mdm2 (Figure 1).** The alignment of the elephant BOX-I sequences of the canonical p53 and the TP53RTGs, illustrates at least two vital substitutions which are conserved in all the TP53RTGs (W>G and N>K). This indicates that these sequences derive from a common initial duplication. The elephant BOX-I Type F isoform has an E to G variation at residue 17. We note that variations on E17 in human p53 are cancer mutants, frequently associated with female genital cancers. **In addition, the cross-species mutations Y>D and E>K, showed an increased docking efficiency.** These variations contribute to the formation of altered p53 BOX-I structures, thus resulting in a range of interaction capacities for these isoforms.

The importance of the results

Making use of a naturally evolved system, the elephant's genome, endogenously expressing a variety of truncated and variable p53 sequences. As such, the **elephant p53 sequences constitute an ideal model for exploring the multifaceted functions of p53.** Our finding provides the structural basis of the p53-mdm2 interfaces, which can be used for the development of highly specific antibodies to target neoantigens and cancer mutations that are difficult to target in conventional ways. Insights into structural modifications and activation toward signaling mechanisms illuminate the factors that prevent or promote carcinogenesis. Our findings on the p53 isoforms can be used to illuminate how their respective activities are regulated in response to cellular damages and pathogen infections. This will not only result in a better understanding of p53 roles and regulation in elephants and in its role in protecting elephants from cancers as well as probably other "inflictions", such as parasites, but it also

facilitates the development of new therapeutic strategies for humans, based on structural molecular data. We envision that our observations and conclusions form a solid basis for further experiments aiming to address how each isoform contributes to the activation of p53, because of structural variability (Figure 1). For the first time such methodology demonstrates how **in silico docking simulations can be used to explore functional aspects of these p53 isoforms** and sets the basis for perspective studies aiming to explore the dynamics of the interactions with mdm2 under stress-inducing conditions. Overall, our study addresses the mechanisms whereby tumor suppressor genes and duplications regulate cellular senescence to drive lifespan and body mass.

(ii) 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.

Objectives of the work

The majority of MHC-I presented peptides are generated in the cytosol by the proteasome and transported into the endoplasmic reticulum (ER) lumen for further processing by the ERAP aminopeptidases, before loaded onto MHC-I molecules by the dynamic multi-component assembly peptide-loading complex (PLC) [22]. The TAP transporters constitute functionally key components of the antigen presentation pathway since they link the cytosolic pool of peptides with the PLC and the ER-resident MHC-I molecules [22]. Impaired TAP function results in reduced surface expression of MHC-I, as the empty MHC-I molecules are unable to present antigens to the immune system and are a common target for viral as well as cancer immune evasion [11]. **Downregulated immune response has been related to impaired TAP expression, which could be a result of structural alterations or dysregulation** [23]. Several **viruses have evolved factors that block the function of TAP** [12], including herpesviruses such as Epstein-Barr virus, human cytomegalovirus, and herpes simplex virus type 1 that establish lifelong persistence in the host. Association of these viruses with certain types of cancer or human malignancies has been demonstrated and knowledge about their targets, as well as functioning mechanisms can be useful in the development of future antiviral treatment strategies. In concert with this, **I investigated the TAP1-TAP2 transporters at the atomic level with a particular focus on how viruses inhibit TAP-mediated peptide translocation at a molecular level.** Molecular dynamics simulations of the following TAP attacking viral immune evasion proteins were investigated; BNLF2a from Epstein-Barr virus, CPXV012 from cowpox virus, ICP47 from herpes simplex virus type 1, US6 from human cytomegalovirus, and UL49.5 encoded by varicellovirus. In addition, we investigated the selectivity of peptides transport by TAPs, among which include peptides derived from the immunopeptidome datasets upon IFN-gamma treatment in melanoma cells. In collaboration with dr hab inż. Umesh Kalathiya (from University of Gdansk, Gdansk), I was involved in **constructing modeled structures for the peptides and predicting their docking affinity / patterns with the TAP transporters.**

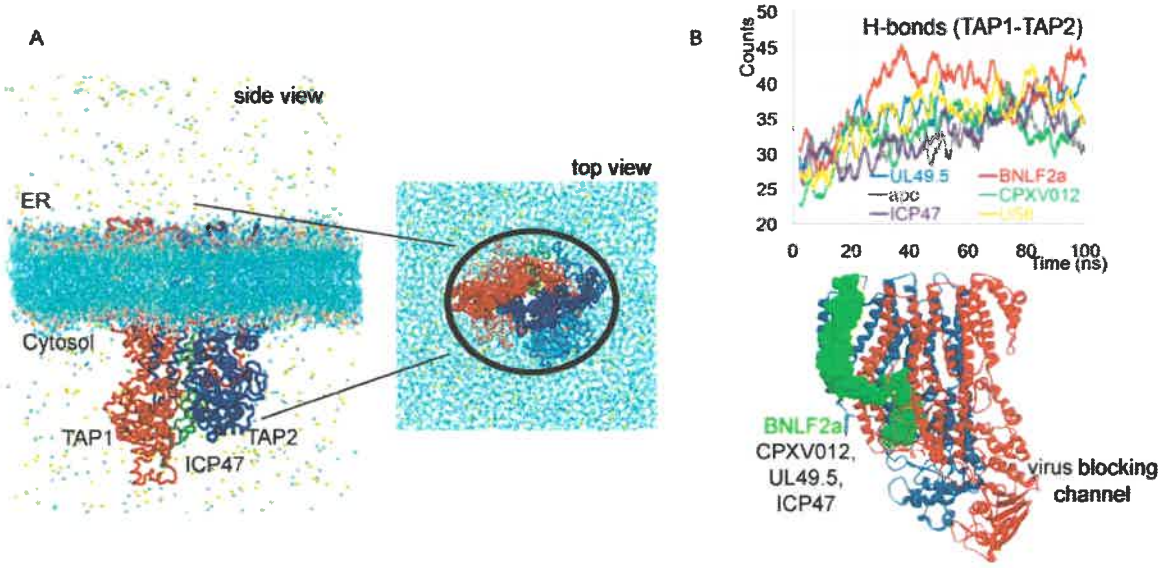

Figure 2. The binding interfaces of different viral proteins (BNLF2a, CPXV012, ICP47, US6, and UL49.5) with the TAP1-TAP2 transporters. **(A)** One molecular dynamic cell in periodic boundary conditions (for example, the TAP1-ICP47-TAP2 complex), which includes proteins, water, and ions. **(B)** The intermolecular hydrogen bond interactions (right panel) formed between the TAP1 and TAP2 proteins during the MD simulation time course and with occupancy > 10%, in the presence and absence of different viral proteins. The below panel shows conformation of viral proteins blocking the transport channel of TAP1/2 transporters.

Description of results

Viral proteins (BNLF2a, CPXV012, ICP47, US6, UL49.5, etc.) can significantly manipulate the peptide transport process by binding directly with the TAP1 or TAP2 transporters. Molecular dynamics datasets revealed that most of the viral protein predominantly destabilizes TAP1, compared to that of TAP2 alone (Figure 2). Particularly, for TAP1 such a visible difference was detected for UL49.5 and BNLF2a, whereas for TAP2 the UL49.5 induced the highest fluctuation and BNLF2a the least. The structural dynamics of the TAP1-TAP2 complex in the presence and absence of a viral protein revealed that, in all **simulated systems the TAP NBD domains formed a tilt movement toward the cytosol membrane.** Such displacement of the TAP NBD domains creates a passage in the transport channel formed by TAP1 and TAP2; opened towards cytosol. From these data it may be proposed that the **opening or closing of the cytosolic passage by the TAP transporters (TAP NBD domains) can be associated with ATP hydrolysis**, and thus, resulting in the peptides being directly presented to the PLC complex. This cytosolic **"open-state" transport channel of the TAP proteins, may intake peptides from cytosol-towards-ER and was targeted by 4 (BNLF2a, CPXV012, ICP47, and UL49.5)** out of 5 studied viral proteins blocking the transport channel (Figure 2). Intermolecular transporter-viral interactions together with the SNPs data and cancer mutations, suggest that in the case of TAP1 protein regions with lower SNPs frequency as well as a few cancer variants were found making higher interactions with viral factors. On the contrary, viral factors bind highly mutated TAP2 regions, and a higher number of residues from **TAP1 were involved in the interactions with viral components**, compared to the TAP2 gene. TAP1 amino acids from helices 4-6, as well as from residue range 1–50 aa located close to the transport channel were found to interact with viral factors.

The importance of the results

Our work highlights the structural and functional insights into the molecular architecture of the peptide transit by the TAP1 and TAP2 transporters, and how cancer-derived mutations as well as different viral factors targeting TAP proteins may suppress the MHC class I pathway. This results in immune escape by the cancer and/or virus-carrying cell. To investigate the kinetics of the peptide transport process, different immunopeptidome datasets were analyzed (MHC-I bound peptides presented by melanoma A375 cells). Viral proteins predominantly destabilize the TAP1 protein, compared to that of the TAP2, particularly, for the UL49.5 and BNLF2a. Among the studied viral proteins, the **UL49.5 protein exhibited the highest fluctuations and BNLF2a was the most stable protein.** The structural dynamics of the TAP1-TAP2 complex in the presence and absence of a viral protein in the complex, revealed that in all simulated systems **the TAP NBD domains formed a tilt movement toward the membrane cytosolic face, which generates a passage or the peptide transport channel open towards the cytosol.** This cytosolic passage was targeted by 4 (BNLF2a, CPXV012, ICP47, and UL49.5) out of 5 studied viral proteins (Figure 2). The BNLF2a, CPXV012, and ICP47 viral proteins formed a higher number of interactions with TAP1, whereas UL49.5 and US6 shared relatively equal interactions with both TAP proteins. In addition, the TAP1 regions with lower **SNPs frequency and cancer variants were found making higher interactions with viral factors**, whereas the viral protein binds to the TAP2 region which is highly mutated. Exceptionally, the US6 protein has unique interactions with the transporters, whereas other BNLF2a/CPXV012/ICP47/ UL49.5 viral factors have several amino acids in common when binding with the TAP proteins. These **results provide a model for how viruses and cancer-associated mutations targeting TAP interfaces can affect MHC-I antigen presentation,** and how the IFN-gamma pathway alters MHC-I antigen presentation via the kinetics of peptide transport.

(iii) Padariya, M., Kalathiya, U., Mikac, S., Dziubek, K., Tovar Fernandez, M. C., Sroka, E., Fahraeus, R., & Sznarkowska, A. (2021). Viruses, cancer and non-self recognition. Open biology, 11(3), 200348.

Viruses have been, and still are, an invaluable source for understanding various aspects of cell biology. The Rous sarcoma virus revealed viral oncogenesis while simian, human adeno (HAdV) and human papilloma (HPV) viruses paved the way for the identification of the p53 and the Retinoblastoma protein (pRB) tumor suppressors [24]. Similarly, **viruses have played an important role in our understanding of how the immune system distinguishes self from non-self,** both in terms of how the innate response detects pathogen patterns and how the adaptive immune system specifically detects the presence of neoantigens on the MHC molecules. Functional viruses that establish a life-long persistence (latency) in humans, such as the gamma herpesviruses Epstein–Barr virus, human cytomegalovirus and Kaposi's sarcoma-associated herpesvirus are present in the majority of the population and each virus has evolved a balance with a specific host cell type [25]. Viral immune evasion strategies also illustrate that processes whereby cells detect and present non-self genetic material to the immune system are interlinked with other cellular pathways. **Immune evasion is also a target for cancer cells and a more detailed look at the interfaces between viral factors and components of the MHC class I** peptide-loading complex indicates that these interfaces are also targets for cancer mutations. In terms of the immune checkpoint, however, viral and cancer strategies appear different.

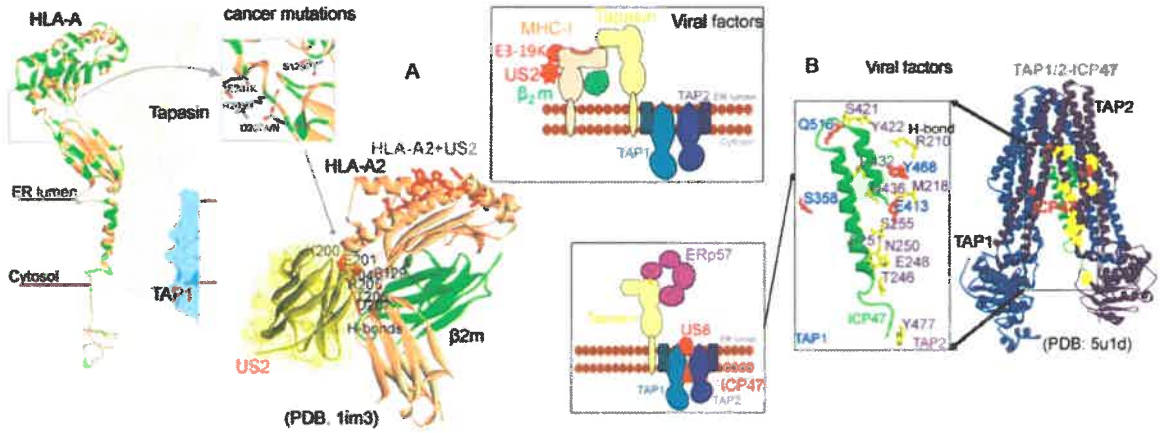

Figure 3. Viral protein binding with different regions of peptide-loading complex compared with the amino acid variants (retrieved from cBioPortal) from different cancer types. **(A)** Mutations in HLA-A from different cancer types (mutations are marked in green color), compared with the viral interaction surface with HLA-A2. Hydrogen bond interactions between the viral proteins US2, US3 and E3/19K with HLA-A2 are marked as red on the protein structure. **(B)** TAP1 and TAP2 cancer mutations, presented as red and yellow, respectively. The viral protein ICP47 residues involved in H-bond interaction with ICP47 from TAP1 and TAP2 are presented as sticks labeled color blue and violet, respectively.

To detect and destroy virus-carrying cells, our immune system also serves to eliminate damaged or harmful cells. **In both viral and cancer immune evasion, the expression of neoantigens on the MHC-I molecules plays a key role (Figure 3)**, and it could therefore be expected that the targets for viral and cancer immune evasion are similar. Indeed, **downregulation of MHC-I molecules is frequent in cancers and a common viral target.** TAP complex is targeted by several viral factors and the ICP47 from the herpes simplex virus locks TAP in an inactive conformation that prevents its role in the PLC [26]. Similarly, the HCMV-encoded US6 glycoprotein interacts with the TAP1 subunit and blocks peptide entry into the ER. Furthermore, the ATP-driven conformational change of TAP1 required for peptide transport from cytosol to ER is the target of the US2 [27]. In order to make a closer examination of the **interfaces between viral interacting proteins and cancer mutations, I constructed complete structures of peptide loading components** (MHC I, β2m, Calreticulin, ERp57, Tapasin, TAP1 and TAP2; Figure 3), which **illustrate common viral and cancer strategies**. The human cytomegalovirus US3 binds to MHC-I in the ER and interferes with tapasin-dependent peptide loading. For **the US6 viral factor, I modeled structure using the homology modeling approach, which was further docked with TAP1/TAP2 transport.**

To locate cancer derived mutations over different **HLA types (HLA-A, HLA-B, and HLA-C) structures, I collected datasets from the cancer genome atlas (TCGA) and the cBioPortal databases (Figure 3)**. The cancer mutations suggest that even though there are similarities between HLA types, several mutations occur only in a particular HLA molecule and in the α3 domain and the transmembrane domains. Furthermore, **the HLA-A molecules have overall more residues mutated as compared to HLA-B and -C.** HLA-C instead shows more mutations in the peptide-binding groove, and it is interesting to note that HLA-C is the major inhibitory ligand for killer immunoglobulin-like receptors (KIRs), which might help to explain why viruses and cancers target this haplotype differently. **A majority of viral proteins interact with regions in the PLC which are highly mutated in cancers.** For example, the viral factors; US2 and E3/19K, form hydrogen bond interactions with the HLA-A2 residues that are mutated in different cancer types. Similarly, UL18 viral factors bind to β2m residues that are

mostly mutated in cancer types. In particular, looking at the TAP transporters from the PLC complex, the viral protein ICP47 forms h-bond interactions with four amino acids (S358, E413, Y468 and Q516) of TAP1 protein, from which two residues (S358F and Q516K) were mutated in cancer. With a glance from these observations of the **viral proteins (US2, E3/19K, ICP47, and UL18) and the cancer mutations, it could be suggested that they share a common interface to interfere with the PLC**.

(iv) 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.

Objectives of the work

Aberrant, misfolded, and mislocalized proteins are dangerous to cell viability due to their toxicity, which can be the cause of multiple human diseases such as Parkinson's and Alzheimer's diseases, frontotemporal dementia, cystic fibrosis, and several others [28]. Particularly at the mRNA level, two essential features are observed: (i) either it contains the correct set of proteins bound to a particular mRNA, or (ii) the coding potential of the mRNA is intact. Here, the NMD (nonsense-mediated mRNA decay) pathway copes with the latter one; by degrading the PTC-containing mRNAs, it consequently reduces the accumulation of potentially toxic truncated proteins [29]. Due to its capacity of **detecting a PTC for accelerating the degradation of the aberrant mRNA, NMD is an important modulator of genetic disease phenotypes in humans** [30]. The **UPF1 protein is a key component in the NMD pathway, also termed as the master regulator.** Through biochemical and single molecule assays, they have shown that a structural element modulates UPF1 catalytic activity, referred to as the 'regulatory loop'. **Interestingly, two alternatively spliced isoforms of UPF1 that differ only in length of the 'regulatory loop' exist in mammals** [31]. UPF1 isoform_1 contains 11 aa (amino acids) insertion (353-GNEDLVIIWLR-363) in domain 1B, which extends the 'regulatory loop' to 22 aa. Considering such influence of the 'regulatory loop' in the structural dynamics of UPF1 and its control over the catalytic and/or ATPase activity, we investigated both isoforms of UPF1 in the presence or absence of the poly(U) mRNA by applying the molecular dynamics simulation technique. Over the trajectories generated in collaboration with dr hab inż Umesh Kalathiya (from University of Gdansk, Gdansk), **I traced hydrogen bond interactions and stability of both UPF1 isoforms (11aa insertion) with poly(U)-mRNA.** In addition, I participated in analyzing **UPF1 A839T and UPF1 P533L/T cancer mutants** influencing the UPF1-mRNA binding (Figure 4). In this study, we investigated different AU or GC rich mRNA motifs with UPF1 protein, and I was involved in generating a set of few mRNA structure models required for the project.

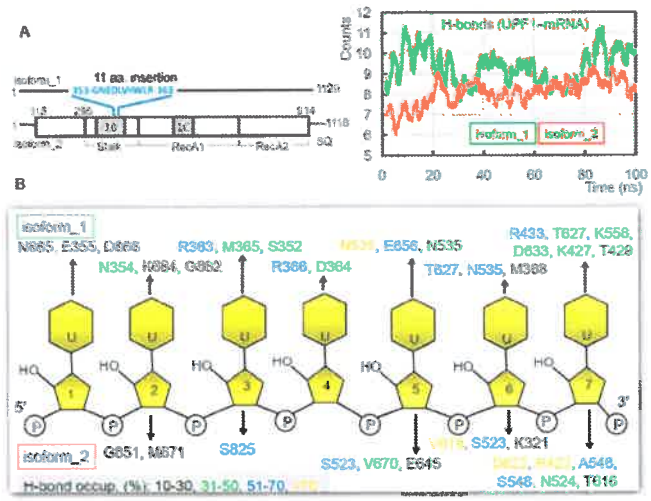

Figure 4. Adopted binding patterns by UPF1 (isoform_1) with different mRNA motifs. **(A)** Frequency of the number of hydrogen bond interactions formed between UPF1 (isoform_1) and different GC-rich or AU-rich motifs. **(B)** Diversity in the binding of UPF1 isoforms with poly(U) mRNA, reflected in their structural dynamics. Individual UPF1 amino acids make long-lasting interactions with the nucleotides having occupancy ≥10%/ns.

Description of results

Binding interfaces and the number of protein–mRNA interactions (hydrogen bond; H-bond) theorized that UPF1 isoform_1 would have a higher number of interactions with poly(U) mRNA compared with that of the UPF1 isoform_2 (Figure 4). **I traced the residues resulting in stable hydrogen bonding with an individual mRNA (occupancy > 10 %/ns)**. The UPF1 isoform_1 was found forming interactions with each nucleotide of poly(U) mRNA, whereas the isoform_2 lacked such a binding pattern. Apart from several isoform-specific UPF1-mRNA interactions, the following amino acids (isoform_2/isoform_1) were common making interactions with mRNAs from both UPF1 isoforms: G851/G862, E645/E656, D622/D633, R422/R433, N524/N535, and T616/T627 (Figure 4). The **presence of 11 aa insertion in the 'regulatory loop' for the UPF1 isoform_1 formed an interface for several residues to form H-bond interactions with the mRNA motifs**—particularly the E355, N354, and R363 residues were involved in such binding. The dynamics of different UPF1 isoforms over MD simulation (100 ns) time, demonstrates that the 11 aa insertion in the 'regulatory loop' induces movements between 1B and RecA2 domains. Point mutation often alters the normal functioning of proteins. Therefore, I participated in investigating such an effect on the UPF1 (isoform_1) helicase activity, upon **inserting the commonly occurring UPF1 cancer variants in the presence of poly(U) mRNA (A839T and P533L/T;** cBioPortal) positioned at the mRNA-binding interface. Upon inserting mutations in the UPF1 protein, a reduction in the protein–mRNA bindings were observed, and such significant changes were traced for the UPF1 P533T system.

The importance of the results

Insertion can induce catalytic and/or ATPase activity, as determined experimentally; however, the kinetics and molecular level information are not fully understood. Alternatively-spliced UPF1 isoforms can bind with different mRNA motifs, and therefore, significant structural dynamics by inserting cancer-derived mutations in the UPF1 mRNA binding pocket was analyzed. In addition, **I constructed a pharmacophore model tracing UPF1-ploy(U) mRNA interactions that could have potential use to screen small molecules blocking the activity of UPF1** protein or eventually the NMD pathway (Figure 4). To this end, our findings suggest that 11 aa insertion (353-GNEDLVIIWLR-363) in the 'regulatory loop' could be responsible for the 'open (isoform_2)' and 'closed (isoform_1)' conformations between 1B and RecA2 domains, which may impact the catalytic activity of the UPF1 helicase. The amino acid positioned at 533 in the UPF1 isoform_1 protein was found interacting with mRNA in the mutated (UPF1 P533L/T) as well as in the wild-type systems, whereas the UPF1 A839T

systems have distinctive interactions with the mRNA motif. Particularly, in **the cancer-associated mutated systems, the mRNA motif with UPF1 P533T lacks the stacking interactions, due to which the distance between 1B-RecA2 domains increased** and the intermolecular protein–mRNA interactions showed a declining trend. From these data it could be proposed that the increased affinity between UPF1-mRNA components should contribute to the enhanced RNA-dependent ATPase/helicase activity of the UPF1 protein that is necessary for the NMD pathway. These novel perspectives from identified mRNA–UPF1 binding pairs can contribute to understanding the selectivity of respective partners, as well as advancing NMD-associated dynamics and kinetics.

(v) Padariya, M., & Kalathiya, U. (2022). The binding specificity of PAB1 with poly(A) mRNA, regulated by its structural folding. Biomedicines, 10(11), 2981.

Objectives of the work

The eukaryotic mRNAs with poly(A) tails bind to sequence-specific poly(A)-binding proteins, mediating synthesis of these tails [32]. One such protein is **poly(A)-binding protein cytoplasmic 1 (PAB1 or PABPC1), associated with the long poly(A) mRNA tails inducing its stability.** The conformational switch of the eIF4G gene depends on the PAB1 expression, i.e., in normal cells having low PAB1 expression, the binding of eIF4G-PAB1 is lacking, whereas in cancer cells with high PAB1 expression, binding is induced between these proteins [33]. Crucial roles of the poly(A)-binding protein for regulation in the translation termination have been investigated, and inefficient termination can result in the activation of the NMD process (initiating mRNA degradation). Different biochemical and structural studies have reported the binding patterns between the RNA recognition motifs with poly(A) mRNA, as well as interaction with different components. However, detailed insights are still needed for the structural folding and dynamics of the full-length human PABPC1 protein. Due to significant correlation between mRNA degradation and stabilization, herein, **I investigated the PABPC1 protein's dynamics, along with tracing mRNA binding specificity.** Additionally, considering different cancer-derived mutations for the PABPC1 protein, I presented an overview of different hotspots [34], along with measuring the change in stability of the structure upon inserting point mutations (Figure 5). In order to monitor the **mRNA selectivity for PAB1 full length structure, I constructed models with the poly(A) mRNA** implementing homology modeling / molecular dynamics approach (Figure 5).

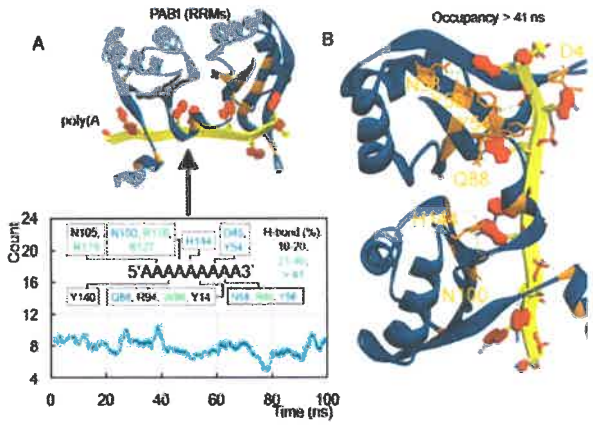

Figure 5. The binding patterns of PABPC1 with the poly(A) mRNA. **(A)** Intermolecular hydrogen bond interactions between PAB1-poly(A) mRNA, as well as individual amino acids interacting with nucleotides having an occupancy of ≥ 10% or nanoseconds (ns), are highlighted. **(B)** Position of high-occupancy (> 41%) residues and their binding pattern with the poly(A) mRNA, represented over the PABPC1 structure.

Description of results

The poly(A)mRNA interacts with the PABPC1, which induces stability within the protein residues. In addition, the modeled PAB1 structure (8–408 aa; RRM1-4 domains) obtained a

pattern of fluctuations like that of other simulated crystal structures consisting of RRM1–2 domains (10–184 aa; Figure 5). In particular, **the poly(A) mRNA was found to be changing its conformations, which stabilized by the end of the MD simulation time (Figure 5)**. Over the modeled apo-form of PAB1, the R176-Y408 residues formed a folded structure after a large displacement, whereas the RRM1–2 domains were found to be conserved and less flexible, almost positioned like those in the crystal structure. **PAB1 region binding with the poly(A) mRNA was more stable and gained a slightly different conformation compared to the apo-form.** The intermolecular interactions between poly(A) mRNA-PABPC1 suggest that ~8 H-bonds were formed every nanosecond (ns), and the PAB1 amino acids were found to be distributed over the mRNA. The PAB1 residues D45, Y54, Y56, N58, Q88, and N100 formed long-lasting interactions having an occupancy >40% with the mRNA. The majority of the PABPC1 point mutations in cancer reside within the RRM domains, and in particular, the regions within 300-350 aa (RRM4 domain), have high mutation frequency. Analyzing the hydrogen-binding residues of the poly(A) mRNA, it was observed that **amino acids engaging in long-lasting interactions were found to be preserved in different cancer types**, i.e., none of them were found to be mutated in the retrieved cancer dataset. Furthermore, mutations with high frequency (>4) were investigated to trace changes in the stability of the protein structure.

The importance of the results

PAB1 is suggested as an antagonist of the NMD factors when targeting mRNA. Despite this, it has been found that **deletion of either PAB1 or UPF1 (NMD) significantly increases the production of novel peptide read-through**. This could result in the increased production of mutant peptides that can be presented over the HLA molecules and could trigger the immune response in cancer. Hence, applying different target inhibition strategies to block the activity of PAB1 completely or partially may result in induced production of peptide read-through over mRNA, which **eventually produces mutant peptide or neoantigens in cancer cells**. A detailed understanding of the PAB1 protein structure can guide such experimental designs to change the activity of this protein. Herein, we investigated the structural folding or dynamics (correlating with the cryo-EM) of the human PAB1 protein, and **proposed several key residues involved in mRNA binding, as well as highlighted different active sites (Figure 5)**. Monitoring the specific mRNA selectivity for PAB1, we reviewed aromatic amino acids (Y or W) associated extensively with the poly(A) mRNA. On the other hand, the poly(A) mRNA has shown a unique pattern towards the PAB1 protein. Every second nucleotide from both the 50 or 30 ends was in an "inward-position" facing towards the protein, whereas every third nucleotide had an "outward position" conformation (Figure 5). The majority of the high-frequency cancer mutations in PAB1 reside within the RRM domains, and in particular, the regions within 300–350 aa (RRM4) have high mutation frequency. However, except for the **G123C variant, the majority of the cancer derived mutants reduced the stability of the protein**. We believe that the molecular details from this study provide a detailed understanding of the PABPC1 structure and can guide future in vitro or in vivo experiments to modulate the activity of this gene.

(vi) 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.

Objectives of the work

SARS-CoV-2 spike (S) gene encodes N-linked 22 glycan sequence per protomer, and these glycans play crucial roles within S protein conformational changes responsible for immune evasion [35]. The **pre-fusion S protein is found comparatively more unstable, the receptor binding domains (RBD) alternate between an "up" (open) and "down" (closed) conformations** [17]. Together the receptor binding domains and N-terminal domains (NTDs) make significant conformational changes inducing flexibility within the structure. Despite different structural approaches to target SARS-CoV-2 virus, there is a lack of a detailed understanding in the direction that would block the homotrimer formation of the SARS-CoV-2 S protein. Considering recent studies on the S protein fusion mechanisms, we sought to **block the homotrimer formation or trapping stabilized pre-fusion conformation by self-derived S peptides or linear motifs.** Since homotrimer is the functional unit of the spike protein, disrupting this homotrimer formation could block the viral entry into the host cell. In this line, **I designed linear motifs that were found involved in the protein-protein intramolecular interaction between monomers of the S homotrimer** and performed molecular dynamics simulations to trace affinity of peptide-protein complexes (Figure 6). Successful blocking the trimer functional unit could have several significant impacts on understanding the SARS-CoV-2; it can induce immune response, a critical component for vaccinology, and a blocked monomer or stabilized homotrimer can induce production of antibody repertoire different from the "open" conformation. Additionally, **I investigated these linear motif self-derived peptides with respect to different mutations derived from the SARS-CoV-2 virus variants** (Alpha | B.1.1.7, Beta | B.1.351, Delta | B.1.617.2 & B.1.617, Gamma | P.1, Lambda | C.37, and Omicron | B.1.1.529) [36, 37].

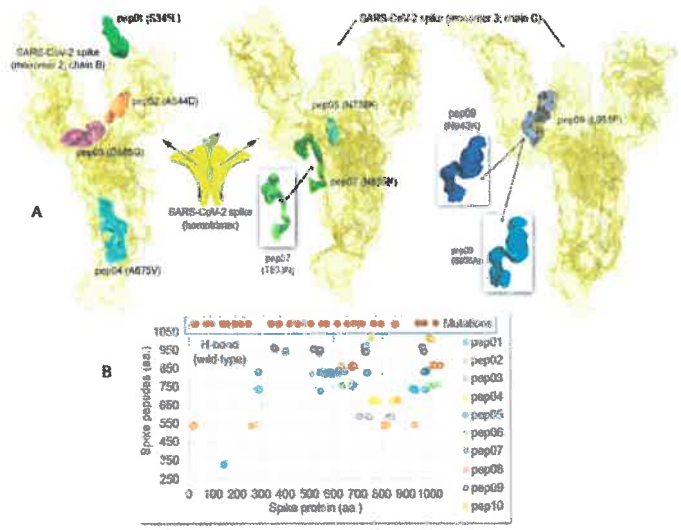

Figure 6. Investigating the influence of different variants over the SARS-CoV-2 spike protein and peptide structures. **(A)** The conformation dynamics upon interesting point mutation in the SARS-CoV-2 S peptides. **(B)** The protein-peptide interacting residues from the wild type systems, compared with the mutations from SARS-CoV-2 variants; Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2 & B.1.617), Gamma (P.1), Lamba (C.37), and Omicron (B.1.1.529).

Description of results

A set of self-derived S peptides from S protein monomer 1 (chain A) were screened against the S protein monomer 2 (chain B), and monomer 3 (chain C). **Peptides belonging in the category of binding with monomer 3 were found showing higher affinity, compared to that of peptides studied with monomer 2 (Figure 6).** The majority of studied mutations (A544D, D588G, A675V, N738K, N830K, T833N, L955F, and S956A) reduce structural stability of the peptide, exceptionally, S371L (pep01) and N943K (pep09) induced a moderate stability within the S peptides. In presence of the S protein, the A544D mutation gained highest instability within its pep02 peptide structure. Particularly, for the pep09 peptide distinct mutations demonstrated a diverse effect on the peptide folding. The structural folding of

differently sized S peptide linear motifs revealed that except the pep02 and pep07 peptides, most of the studied peptides have obtained a high conformational movement with respect to their starting positions (Figure 6). Stable (pep02 and pep07) **peptides were found showing a higher number of interactions with the S protein that induced stable shaping or folding in the S protein structure**. For example, a contrast conformation within the S protein is traced when put together with a peptide having less binding affinity (pep01), compared to that with the pep07 peptide. Regions of the S protein ranging 250–450, 680–760, and 915–1000 has induced stability for the S protein in presence of the pep02 and pep03 peptides, whereas contrast behavior was observed for the pep01, pep04, and pep05 peptides. Overall, for the **mutant peptide system a higher number of residues were found binding the S protein, despite increased flexibility in the protein structure (Figure 6)**. Overlaying S protein mutation from SARS-CoV-2 variants with the protein-peptide interactions from the wild type system, highlighted that most mutations reside in regions where self-derived peptides interact with the S protein.

The importance of the results

Several structural based approaches have already been applied to develop vaccines targeting the SARS-CoV-2 virus, however, there lacks a detailed understanding in the direction to block the homotrimer formation of the S protein. Herein, we propose that **disruption in homotrimer formation may block the viral entry into the host cell or reduce the kinetics of the S protein**. Molecular dynamics data revealed that a larger set of S protein residues ranging from 550-750 and 950-1050 in the wild type systems were found interacting with different self-derived S peptides (Figure 6). Peptides pep02 and pep07 in the wild type systems were among high affinity binders with the S protein, and in particular, the point mutation in the pep02 peptide lowered the number of protein-peptide interactions. Moreover, the **A544D mutation from the pep02 peptide induced instability for the complex S protein, whereas the point mutation N943K from pep09 had exhibited a contract behavior**. The presence of mutation in the system has induced an "up" active conformation of the spike (RBD) domains that could enhance the interaction with the host cell receptor. Inserting mutation in the peptides destabilized the complexed S protein structure, which resulted in the allosteric effect over other different functional regions of the protein. The **frequency of mutations in the protein-protein binding affinity changes within the trimeric S protein were identified**. The A544R (pep02) mutation can have a significant impact on the binding affinity within the spike monomers of the homotrimer unit. We believe that our findings can allow better understanding of the SARS-CoV-2 spike homotrimer, and our data could help to design further experiments to develop self-derived peptides replacing a single S protein monomer from the trimer, blocking the homotrimer functional unit or inducing stability.

(vii) 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.

Radiotherapy is an effective treatment for many cancer types, which is used in ~60% cancer patients and frequently with surgery or chemotherapy. A decade of effort has demonstrated that the Type 1 interferon (IFN-I) cytokine system is compelling in mediating the efficacy of radiotherapy [38]. DNA damage can trigger innate immune response through the accumulation of nuclear DNA in the cytoplasm, a common characteristic of tumors and cancer cell lines in the accumulation of cytoplasmic ssDNA or dsDNA [39]. Radiotherapy and chemotherapy are

the standard treatments for beating cancer, but tumor resistance to these methods is raising a great concern. Interferon-stimulated gene expression encompasses an IFN-related DNA damage resistance signature or IRDS, which is strongly associated with resistance to radiation and chemotherapy across different tumors. **Clinical data from different breast cancer datasets highlighted a correlation between sensitivity to chemotherapy and low expression of IRDS genes** [40], also corroborated in glioblastoma dataset [40]. Additionally, the radiation in breast, prostate, and glioma cancer cells also elevates IRDS gene expression [41]. Several efforts are being made to understand how the IRDS genes protect malignant cells from eradication. Particularly, the STAT1/IFN pathways are responsive to DNA damage, and in a healthy state, there is no free DNA or RNA released in the cytosol, whereas upon pathogen attack the exogenous DNA is recognized by the Type I IFN.

The mechanisms by which IFNs promote the efficacy of standard-of-care cancer treatments, remains an area of active investigation. According to antiviral potency, several studies ranked the IFN subtypes, with IFNa8 often being the most potent and IFNa1 up to 1000-fold less potent. In this review paper, **I outlined different strategies adopted to suppress these IRDS genes (for example, STAT1, IRF7, OAS family and BST2) in cancer inducing the sensitivity (chemo- and radiotherapy)**, as well as different ways by which the viruses inhibit the IRDS genes are addressed. In addition, **major upstream regulators (IRF7, STAT1, EIF2AK2, IFIH1, USP18, ISG15, DCN, IFIT1 and TIMP3) from the IRDS gene set were identified by me**, and different IFNs regulating these genes were identified (Figure 7). In order to interpret the involvement of IRDS genes in different biological processes, **I performed pathway enrichment analysis combining g:Profiler and Cytoscape (Figure 7)**. In total, thirty different biological pathways associated with either of the IRDS genes were classified, and from that 29 pathways were clustered as the 'interferon viral regulation'. Seven highly populated biological pathways with the IRDS genes were identified, and with recording their frequently associated genes (IFIT1/3, IFITM1, IRF7, ISG15, MX1/2 and OAS1/3/L; Figure 7).

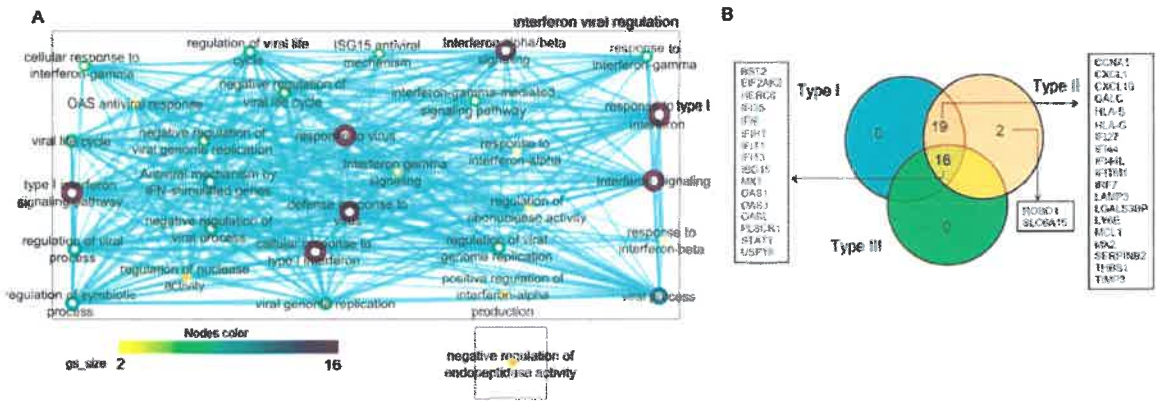

Figure 7. Differentiating IRDS genes associated significant biological pathways. **(A)** Pathway enrichment analysis for the IRDS genes performed using g:Profiler, and as the following step, the networks were visualized using the "Enrichment Map" platform in the Cytoscape package. **(B)** Majority of the IRDS genes were found regulated by both IFNs Type I and II or by all three IFN types, whereas only a small number of genes are regulated by a single type; Type II IFN. The pie chart is generated considering the experimental datasets from the Interferome database.

Moreover, to identify major regulators among the IRDS genes, we performed upstream regulator analysis employing protocols from the ingenuity pathway analysis (QIAGEN-IPA).

The data highlighted the following genes as the upstream regulators; IRF7, STAT1, EIF2AK2, IFIH1, USP18 (ubiquitin specific peptidase 18), ISG15, DCN (decorin), IFIT1, and TIMP3. Both Type I and Type II IFNs regulate 19 IRDS genes and 16 genes were found regulated by all three IFN types, whereas ROBO1 and SLC6A15 genes are only regulated by Type II IFN (Figure 7). These IRDS genes are identified to be induced in diverse cancer cell lines in response to chemo- and radiotherapy or mediate experimental resistance, as well as signatures have been traced in cancer patients samples that correlate with the resistance. Silencing of these genes resensitizes triple negative breast cancer (TNBC) cells to chemo- and radiotherapy in vitro and in vivo, illustrating the potential therapeutic power of modulating this response [42]. Considering the importance of such active amino acids, herein, **I reported different possible functional active sites of IRDS genes based on their available tertiary structures** or proposed them by homology modelling approach (Molecular Operating Environment, MOE; Chemical Computing Group Inc., Montreal, QC, Canada).

IRDS genes were distinguished as DNA or RNA binders, and those essential for binding to nucleotide ATP/GTP/NADP molecules. To capture novel insights within **IRDS genes we further investigated their tertiary structures, providing a glance of functional interfaces**. Like the STAT1 and IRF7 proteins forming a well-defined pharmacophore active site with dsDNA, the genes OAS1, OAS3, and IFIH1 defined their pharmacophore with dsRNA. Lys residue was found common in all three genes (OAS1, EIF2AK2, and IFIH1) binding with the phosphate groups of the ATP molecule. Additionally, the MX1 and HSD17B1 genes defined a conserved active site model with the GDP and NADP+ molecules, respectively. These details revealing the **IRDS genes can be of immense importance, as a predefined active site is always effective to classify target specific molecules**. In particular, structural knowledge can support different strategies to target the identified functional sites in IRDS genes and may open doors for several genes that have not fundamentally validated for such applications.

(viii) 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.

Objectives of the work

Solvents play an important role in the structure, dynamics, and function of biological systems. The structure and dynamics of water molecules in the vicinity of the proteins absolutely depend on the nature of proteins such as hydrophilicity or hydrophobicity, which is the main factor of thermodynamic stability of folded proteins in aqueous solutions [43]. A deeper understanding of the solvation phenomena of biomolecules in aqueous and non-aqueous solutions is crucial. Majority of **enzymatic catalytic reactions take place in aqueous solutions, some can happen in non-aqueous solutions of organic solvents (OS), ionic liquids (ILs) or deep eutectic solvents (DESs)** due to low solubility of reactants or products in aqueous solutions [44]. Herein, as an example, **I focused on measuring changes in the active site of protease [45]**, as due to lack of water molecules to solvate the active site when enzymes are placed in highly concentrated solutions of OS and ILs. The role of water molecules in the structure, dynamics, and function of biomolecular systems is important especially in low water content solutions, hence, we investigated how much water content is needed in enzymatic catalytic reactions in aqueous solutions of organic media. **I constructed different in silico model systems for the protease enzyme in the presence or absence of Darunavir drug, as well as performed by a range of molecular dynamics simulations where deep eutectic solvents (DESs) differ by organic solvents or concentration**. The following four different

organic solvents; choline chloride (CHL), ethylene glycol (EG), glycerol, and urea were considered with a focus on behavior of protease active site and changes in ligand bindings when present in different solvent environments.

Description of results

The MD simulations on different protease model systems, guided to understand effects of the aqueous solutions of organic solvents on the protease enzyme's structural activities. **Over the simulated protease enzyme in different conditions and organic solvents (choline chloride / ethylene glycol / glycerol / urea), I measured the stability of individual monomers** of protease homodimer with the Darunavir molecule. The protease enzyme in the choline chloride + urea aqueous solution has shown greater stability, compared when in presence of other studied solvents. Enzyme with the Darunavir, appeared to be a stable structure compared to the apo-form (CHL + urea). A similar behavior was observed for Darunavir, i.e., ligand is less flexible in the CHL + urea, compared to other studied aqueous solutions. Distinctive behavior of protease explains the effect of organic solvent over the structure folding whereas, both protease monomers with Darunavir have obtained a higher number of stable amino acids compared to the systems without ligands in the CHL + urea aqueous solution. Particularly, **residues ranging from 45 to 55 amino acids from the 'flaps' were found to be more flexible in the system without ligands**. Overall, the presence of Darunavir has induced the protein-protein intermolecular interactions between two protease monomers in different aqueous solutions. In the model systems with **CHL + EG and CHL + glycerol organic solvents, a declining trend of protein-protein interactions was observed over 500 ns of MD simulation time**. Clustering of organic solvents was observed for the systems containing choline chloride + ethylene glycol or choline chloride + glycerol. Specifically, ethylene glycol and glycerol were found forming clusters in the presence of choline chloride (1:2 ratio making 20% of solvent in the simulation box) and water molecules. This self-clustering induced an 'open' conformation for the flap domains of the protease, in the presence or absence of the ligand. Lacking aggregation in the CHL + urea aqueous solution, the protease enzyme showed conventional structural folding.

The importance of the results

Alteration in the enzymatic activity, bio-catalysis selectivity or enzymatic stability can be obtained by changing the micro-solvation environment of the enzymes or by other solvents than native ones. **Proteases in the CHL + urea aqueous solution showed a greater stability compared to when with other solvents (CHL + EGl or CHL + glycerol)**. Darunavir ligand induced protein-protein interactions between monomers of protease in different aqueous solutions. The majority of residues involved in such interactions come from the whiskers, eye, and nose domains of the protease enzyme. Exclusive for the systems containing urea ILs, both monomers were actively involved in the interaction with the Darunavir molecule. In systems with the CHL and urea, the D25 and G27 amino acids conserved among the aspartic protease enzymes were found binding with Darunavir. In addition, the **residues R8, D30, G48, and I50 were found to be common binding the Darunavir ligand in the presence of different organic solvents**. Our findings suggest that self-aggregation within a particular type of organic solvent has a significant effect over the folding of the protease and its binding with Darunavir. Hence, to maintain such nonpeptidic inhibitor activity towards the protease enzyme organic solvents can have a significant influence, and our current findings can guide further in vitro or in vivo experiments in this direction. However, we believe that our novel findings shall help to better understand the HIV-1 life cycle maintained by protease

enzymes, and its interactions with inhibitors, which could be controlled by deep eutectic solvents.

Summary of the most important achievements

1. Exploring functional aspects of p53 isoforms and their dynamics of the interactions with mdm2

The p53–mdm2 pathway has coevolved, with mdm2 remaining largely conserved, whereas the TP53 gene morphed into various isoforms. I modeled initial structures of the BOX-I motifs of the elephant p53 isoforms in order to test the hypothesis that p53 isoforms induce distinct pools of p53 proteins with variations on the epitopes interacting with mdm2. I implemented the homology modeling approach and constructed the elephant mdm2 structure. A specific set of elephants p53 isoforms were docked over the mdm2 E3 Ubiquitin Ligase by me, in order to identify the binding affinity and conformation pattern of p53 gene (elephant L. african). Retrogene 12 along with retrogenes 14, 15, 16, and 18, form the group "Type F," which shows poor binding to mdm2 strongly indicating that these putative proteins escape mdm2-dependent regulation. In addition, our in silico analysis and the experimental sandwich ELISA findings both show that all the peptides carrying the W>G variation exhibit decreased binding activity with mdm2. Moreover, the cross-species mutations Y>D and E>K, showed an increased docking efficiency.

2. Investigating viral factors targeting different sites of TAP1-TAP2 transporters, along with a focus on how viruses inhibit TAP-mediated peptide translocation at a molecular level

Viral proteins can significantly manipulate the peptide transport process by binding directly with the TAP1 or TAP2 transporters. Therefore, to understand the molecular recognition of the TAP1-Virus-TAP2, and particularly the different viral proteins I simulated their interactions with the TAP transporter. Viral proteins predominantly destabilize the TAP1 protein, compared to that of the TAP2, particularly, for the UL49.5 and BNLF2a (Figures 2 and 8). Among the studied viral proteins, the UL49.5 protein exhibited the highest structural fluctuations and BNLF2a was the most stable protein structure. TAP1 protein regions with lower SNPs frequency as well as a few cancer variants were found making higher interactions with viral factors. On the contrary, viral factors bind highly mutated TAP2 regions. Long lasting interactions (> 10 ns) between TAP-viral proteins were identified, defining a pharmacophore model (Figure 8). The cytosolic "open-state" transport channel of the TAP proteins may intake peptides from cytosol-towards-ER and was targeted by studied viral proteins (Figure 8). In addition, I was involved in constructing modeled structures for the peptides and predicting their docking affinity / patterns with the TAP transporters.

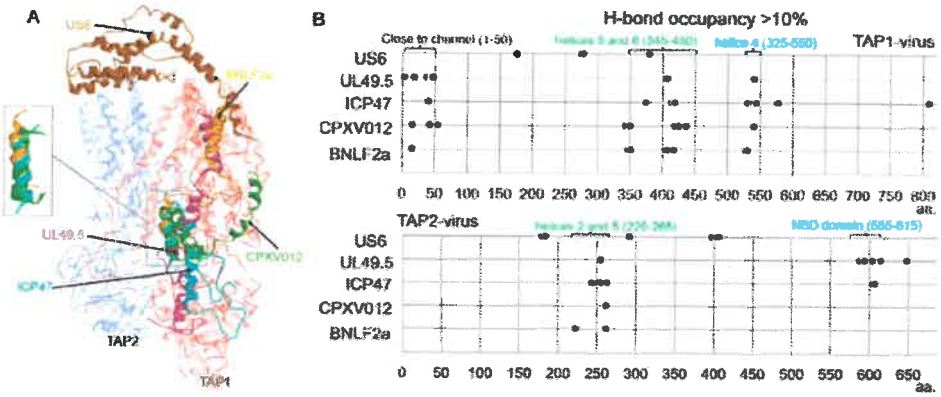

Figure 8. Screening of viral proteins, peptides, and cancer-associated mutations on the TAP1-TAP2 transporters. **(A)** Viral proteins (BNLF2a, CPXV012, ICP47, US6, and UL49.5) docked with the TAP transporters, and the binding conformations for each studied viral component resembles almost a complementary state. **(B)** Residues from TAP1 or TAP2 are involved in binding viral proteins with occupancy 10%, and the right panel represents the position of these regions over the protein structure.

3. Constructing the pharmacophore model based on the interactions of two UPF1 isoforms with poly(U)-mRNA

Due to its capacity of detecting a PTC for accelerating the degradation of the aberrant mRNA, NMD is an important modulator of genetic disease phenotypes in humans. The UPF1 protein is a key component in the NMD pathway, also termed as the master regulator. Interestingly, two alternatively spliced isoforms of UPF1 that differ only in length of the 'regulatory loop' exist in mammals. I traced hydrogen bond interactions and stability of the UPF1 both isoforms (11 aa insertion) with poly(U)-mRNA. The presence of 11 aa insertion in the 'regulatory loop' for the UPF1 isoform_1 formed an interface for several residues to form H-bond interactions with the mRNA motifs—particularly the E355, N354, and R363 residues were involved in such binding. G851/G862, E645/E656, D622/D633, R422/R433, N524/N535, and T616/T627 amino acids (isoform_2/isoform_1) were common making interactions with mRNAs. In addition, I was involved in analyzing UPF1 A839T and UPF1 P533L/T cancer mutants influencing the UPF1-mRNA binding.

4. Investigating the PABPC1 protein's binding specificity with tracing poly(A)-mRNA, a set of preserved protein-mRNA in different cancer were identified

The poly(A)-binding protein cytoplasmic 1 (PAB1 or PABPC1) protein is associated with the long poly(A) mRNA tails, inducing stability. Due to significant correlation between mRNA degradation and stabilization, herein, I investigated the PABPC1 protein's dynamics, along with tracing mRNA binding specificity. To monitor the mRNA selectivity for PAB1 full length structure, I constructed models with the poly(A) mRNA implementing homology modeling. The poly(A) mRNA was found to be changing its conformations, which stabilized by the end of the MD simulation time. Over the modeled apo-form of PAB1, the R176-Y408 residues formed a folded structure after a large displacement, whereas the RRM1–2 domains were found to be conserved and less flexible. PAB1 region binding with the poly(A) mRNA was more stable and gained a slightly different conformation compared to the apo-form. Long-lasting interactions were found to be preserved in different cancer types, i.e., none of them were found to be

mutated in the retrieved cancer dataset. Except for the G123C variant, the majority of the cancer derived mutants reduced the stability of the protein.

5. Self-derived linear motifs based on intramolecular interaction between monomers of the SARS-CoV-2 spike homotrimer

The structural spike protein from the SARS-CoV-2 β-coronavirus is shown to make different pre- and post-fusion conformations within its homotrimer unit (Figure 9). To support the ongoing novel vaccine design and development strategies, and to block the homotrimer formation or trapping stabilized pre-fusion conformation I designed self-derived S peptides or linear motifs. The majority of studied mutations (A544D, D588G, A675V, N738K, N830K, T833N, L955F, and S956A) reduce structural stability of the peptide, exceptionally, S371L (pep01) and N943K (pep09) induced a moderate stability within the S peptides (Figure 9). In presence of the S protein, the A544D mutation gained highest instability within its pep02 peptide structure. Stable (pep02 and pep07) peptides were found showing a higher number of interactions with the S protein that induced stable shaping or folding in the S protein structure. Additionally, I investigated these linear motif self-derived peptides with respect to different mutations derived from the SARS-CoV-2 virus variants. In the mutant peptide system, a higher number of residues were found binding the S protein, despite increased flexibility in the protein structure.

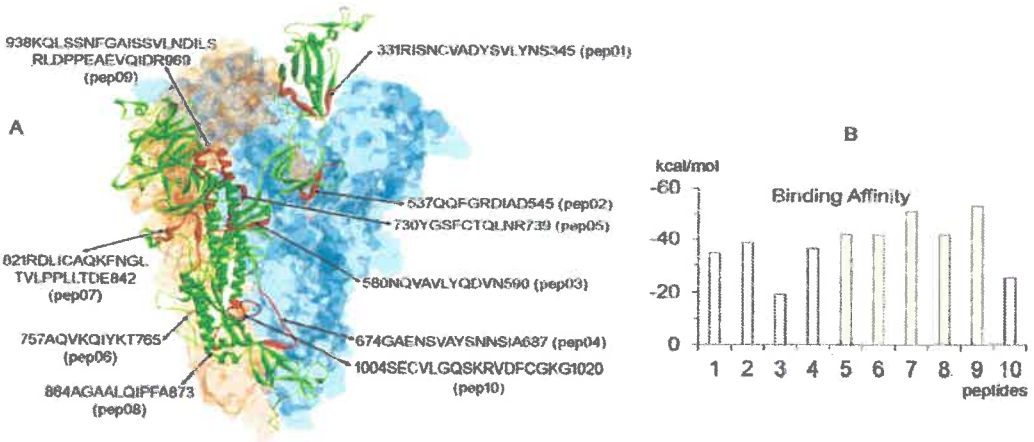

Figure 9. Recognized self-derived SARS-CoV-2 spike peptides linear motifs, and their binding affinity with the spike protein (monomers). **(A)** Considering different possible intermolecular interactions between spike monomers of the homotrimer unit, a set of 10 different self-derived peptides were constructed. **(B)** The binding affinities of 10 linear motif peptides (from monomer 1; chain A) with their respective S protein monomer.

6. Different functional interface of IRDS genes, with providing a glance of their involvement in different biological processes

Clinical data from different breast cancer datasets highlighted a correlation between sensitivity to chemotherapy and low expression of IRDS genes. Considering the importance of these genes, I outline different strategies adopted to suppress these IRDS genes (for example, STAT1, IRF7, OAS family and BST2) in cancer-inducing sensitivity. In addition, major upstream regulators (IRF7, STAT1, EIF2AK2, IFIH1, USP18, ISG15, DCN, IFIT1, and TIMP3) from the IRDS gene set were identified. Thirty different biological pathways associated with either of the IRDS genes were classified, and from that 29 pathways were clustered as the

'interferon viral regulation'. I reported different possible functional active sites of IRDS genes based on their available tertiary structures or proposed them by homology modeling approach.

7. Measured changes in the active site of the enzyme in highly concentrated solutions of deep eutectic solvents

Enzymatic catalytic reactions take place in aqueous solutions, some can happen in non-aqueous solutions of organic solvents, ionic liquids, or deep eutectic solvents due to low solubility of reactants or products in aqueous solutions. As an example, I focused on measuring changes in the active site of protease. I constructed different in silico model systems for the protease enzyme in the presence or absence of Darunavir drug, as well as performed a range of molecular dynamics simulations where deep eutectic solvents (DESs) differ by organic solvents or concentration. The protease enzyme in the choline chloride + urea aqueous solution has shown greater stability, compared when in presence of other studied solvents. For CHL + EG and CHL + glycerol organic solvents, a declining trend of protein-protein interactions was observed. The residues R8, D30, G48, and I50 were found to be common binding to the Darunavir ligand in the presence of different organic solvents.

Scientific plans

I am actively involved in the project entitled: "The impact of UPF1 ATP mimetics on the mutant immunopeptidome", funded by National Science Center (in 2021) under the OPUS program and led by Prof. Ted Hupp at University of Gdansk. The data generated in my publications 4.2, 4.3, and 4.4 became the basis for this project and grant application. Small-molecule inhibitors that can modulate NMD activity offer critical tools for understanding the mechanism and physiological functions of the NMD pathway, and they also have the potential for treating certain genetic diseases and cancer. The innovative aspects of the proposed project involve the development of new anticancer inhibitors/molecules that will act on completely a new target, namely hUPF1. In addition, the project originates from an unmet clinical need due to the inefficiency of existing cancer therapeutics as well as high and inadequate costs of biological therapies including high costs of patient-specific genetic vaccines. By contrast, inhibiting hUPF1 with a small molecule, generating translation readthrough thus creating neoantigen by virtue of the amino acid which is added at a stop codon, in a sense stimulates the tumor cell to make its own neoantigen vaccine. I will be implementing molecular modeling and structure-based virtual screening (SBVS) methods to select promising compounds from available chemical databases and design derivatives active against UPF1 gene. In addition, I will be involved in performing analysis of proteomics datasets or the immunopeptidome data obtained from mass spectrometry and predict their binding affinity or kinetics with TAP transporters.

In the 2023 year, I participated as co-investigator in the project entitled: Specificity in detection of PTCs in mRNA by NMD and its network, insights from cancer perspective and cross-linking (XL-MS), led by dr hab. inż Umesh Kalathiya (under the SONATINA, National Science Center, program). I investigated the SURF complex (SMG1-UPF1-eRF1-eRF3) components by modeling SMG1, SMG8, SMG9, eRF3a, eRF1, and UPF1 proteins along with investigating their molecular dynamics and interaction networks. The SURF complex binds with the UPF2, UPF3b and an EJC downstream of the PTC, forming the decay-inducing complex (DECID). Along with the UPF proteins the SURF complex promotes the phosphorylation of UPF1 by SMG1. In addition, I identified binding specificity of eIF4A3 to mRNA, performing docking of

EIF4A3 with distinct mRNA fragments. As the continuation of my participation in this project, I will be involved at different stages of analysis of data retrieved from molecular dynamics and cross-linking mass spectrometry approaches.

In cancer, the p53 gene exhibits some classical features of a tumor suppressor including loss of heterozygosity and it is distinguished by the frequency of missense mutations found in the gene. Indeed, the majority of mutations (>70%) are single amino acid (missense mutations) that generate a defective and abundant protein. This latter fact is highly important to anti-cancer drug researchers as it allows p53 to be potentially targetable. Approximately 80% of p53 mutations are point mutations with several hotspot mutations having a dominant-negative effect on wild-type p53 activity. Beyond this, p53 mutants also acquire new oncogenic functions. These gain of function mutants are highly associated with late-stage malignance, drug resistance and chemoresistance, for example through the p53 mediated targeting of MDR1 (multidrug resistance gene 1) [46]. Moreover, the mdm2 E3 ubiquitin ligase interacts with the p53 as the negative regulator of tumor suppressor p53, in this direction, I will be working on identifying novel drug molecules blocking p53-mdm2 network through targeting mdm2 active sites. In addition, I am planning and designing the project / grant application to optimize novel oligonucleotides binding mdm2, that can induce an allosteric regulation over mdm2-p53 interactions. Additionally, the structural folding of different p53 mRNAs will be investigated with the mdm2 gene, along with tracing their dynamics.

As a continuation of my interest in viral proteins targeting the structure and dynamics of peptide-loading complex (PLC) components that result in impairment of MHC-I antigen presentation, I will generate complete structure PLC complexes and perform molecular simulations with different viral factors. Hyper polymorphic MHC-I molecules are key components for acquired immune responses, which have high specificity towards peptides presented to $CD8^+$ T cells (resembling the vaccine design and autoimmunity). However, a broader landscape of structural differences (plasticity of ectodomains along with transmembrane region) of these genes remains elusive, and therefore, applying a consensus 3D homology modeling approach I will be involved in investigating a large dataset of HLA-A, -B, -C polymorphic structures. In addition, a family wide comparison of different allele coordinates highlighting a set of few alleles with structural diversity in the peptide-loading groove will be performed. My project entitled: Quantifying binding and interaction dynamics of peptide-loading complex components upon viral interference, received funding under UGrants-START. This program from University of Gdansk, Gdansk supports the research implementation and covers publication costs.

5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

Description of scientific work

My master's thesis project was entitled: "Computer-aided design of Organophosphorus inhibitors of Urease", under the supervision of prof. dr hab. inż. Łukasz Berlicki and prof. dr hab. inż. Wacław Andrzej Sokalski. I received my Master of Science and Engineering (mgr inż.) studies in Biotechnology / Bioinformatics field in 2011 at Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland. Considering the most potent inhibitor

(diamidophosphate) several compounds designed with covalent carbon-phosphorus or carbon-phosphorus-carbon bond to improve hydrolytic stability to inhibit the microbial ureases.

- **Padariya, M.**, Kalathiya, U., Berlicki, L., & Baginski, M. (2014). Computer-aided design of organophosphorus inhibitors of urease. International Journal for Computational Biology, 3(1), 31-38.

In 2013 year, I started my doctoral studies, and my project was entitled: "Structural and dynamic insights on the EmrE protein in apo-form and with TPP+ related substrates". I received a Ph.D. (dr inż.) or doctoral degree in November 2018 in chemical sciences in the field of Biotechnology, at the Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology in the group of prof. dr. hab. inż. Maciej Bagiński. Prof. Marco Mor (Medicinal Chemistry Food and Drug Department, University of Parma, Parma Italy) and dr hab. Krzysztof Murzyn (Wydział Biochemii, Biofizyki i Biotechnologii, Uniwersytet Jagielloński, Kraków, Poland), were reviewers of my doctoral thesis. During my doctoral studies I was a member of the Europin PhD Program (2014-2018; https://www.univie.ac.at/europin). My research direction during doctoral studies was focused on characterizing the molecular properties of the small MDT protein EmrE (ligand recognition, protein conformation change, and ligand movement) using the MD simulation approach. First, to understand how this protein may recognize ligands, the monomer and asymmetric apo-form of the EmrE dimer were embedded in a heterogeneous phospholipids membrane, and second, the EmrE protein in the complex with substrate or ligand molecule TPP^+, and its derivatives were studied.

- **Padariya, M.**, Kalathiya, U., & Baginski, M. (2015). Structural and dynamic changes adopted by EmrE, multidrug transporter protein-studies by molecular dynamics simulation. Biochimica Et Biophysica Acta (BBA) - Biomembranes, 1848(10), 2065-2074. doi:10.1016/j.bbamem.2015.05.014; (**IF_{2015} 3.687; $MEiN_{2015}$ = 35,** according to the new MEiN scores **$MEiN_{2022}$ = 100**).

- **Padariya, M.**, Kalathiya, U., & Baginski, M. (2018). Structural and dynamic insights on the EmrE protein with TPP^+ and related substrates through molecular dynamics simulations. Chemistry and Physics of Lipids, 212, 1-11. doi:10.1016/j.chemphyslip.2017.12.004; (**IF_{2018} 2.536; $MEiN_{2018}$ = 25,** according to the new MEiN scores **$MEiN_{2022}$ = 100**).

Along with my main doctoral project, I actively participated in several collaborative projects in the department. My expertise in applying computational and theoretical methods for understanding biomolecular systems: computer-aided drug design, molecular modeling, molecular docking, and molecular dynamics simulation, enhanced several projects (13 research papers) to assist in reaching their scientific goals.

- Kalathiya, U., **Padariya, M.**, & Baginski, M. (2018). The structurally similar TRFH domain of TRF1 and TRF2 dimers shows distinct behaviour towards TIN2. Archives of Biochemistry and Biophysics, 642, 52-62. doi:10.1016/j.abb.2018.02.005; (**IF_{2018} 3.559; $MEiN_{2018}$ = 30,** according to the new MEiN scores **$MEiN_{2022}$ = 100**).

- Kalathiya, U., **Padariya, M.**, & Baginski, M. (2018). Extracting functional groups of ALLINI to design derivatives of FDA-approved drugs: inhibition of HIV-1 integrase.

Biotechnology and Applied Biochemistry, 65(4), 594-607. doi:10.1002/bab.1646; (**IF_{2018} 1.559; $MEiN_{2018}$ = 20,** according to the new MEiN scores **$MEiN_{2022}$ = 40**).

- Kalathiya, U., **Padariya, M.**, & Baginski, M. (2017). Molecular basis and quantitative assessment of TRF1 and TRF2 protein interactions with TIN2 and Apollo peptides. European Biophysics Journal with Biophysics Letters, 46(2), 171-187. doi:10.1007/s00249-016-1157-7; (**IF_{2017} 1.935; $MEiN_{2017}$ = 20,** according to the new MEiN scores **$MEiN_{2022}$ = 70**).

- **Padariya, M.**, & Kalathiya, U. (2017). Comparative molecular dynamics study of dimeric and monomeric forms of HIV-1 protease in ligand bound and unbound state. General Physiology and Biophysics, 36(2), 141-154. doi:10.4149/gpb_2016028; (**IF_{2017} 1.479; $MEiN_{2017}$ = 15,** according to the new MEiN scores **$MEiN_{2022}$ = 40**).

- **Padariya, M.**, Kalathiya, U., & Baginski, M. (2017). Molecular basis and potential activity of HIV-1 reverse transcriptase toward trimethylamine-based compounds. Biotechnology and Applied Biochemistry, 64(6), 810-826. doi:10.1002/bab.1543; (**IF_{2017} 1.44; $MEiN_{2017}$ = 20,** according to the new MEiN scores **$MEiN_{2022}$ = 40**).

- Kalathiya, U., **Padariya, M.**, & Baginski, M. (2016). Identification of 1H-indene-(1,3,5,6)-tetrol derivatives as potent pancreatic lipase inhibitors using molecular docking and molecular dynamics approach. Biotechnology and Applied Biochemistry, 63(6), 765-778. doi:10.1002/bab.1432; (**IF_{2016} 1.413; $MEiN_{2016}$ = 20,** according to the new MEiN scores **$MEiN_{2022}$ = 40**).

- **Padariya, M.**, & Kalathiya, U. (2016). Structure-based design and evaluation of novel N-phenyl-1H-indol-2-amine derivatives for fat mass and obesity-associated (FTO) protein inhibition. Computational Biology and Chemistry, 64, 414-425. doi:10.1016/j.compbiolchem.2016.09.008; (**IF_{2016} 1.331; $MEiN_{2016}$ = 20,** according to the new MEiN scores **$MEiN_{2022}$ = 70**).

- Kalathiya, U., **Padariya, M.**, Baginski, M., & Padariya, C. (2015). SiMiSnoRNA: collection of siRNA, miRNA, and snoRNA database for RNA interference. Turkish Journal of Biochemistry, 40(6), 524-532. doi:10.1515/tjb-2015-0044; (**IF_{2015} 0.211; $MEiN_{2015}$ = 15,** according to the new MEiN scores **$MEiN_{2022}$ = 20**).

- **Padariya, M.**, Kalathiya, U., & Baginski, M. (2014). Docking simulations, molecular properties and ADMET studies of novel chromane-6,7-diol analogues as potential inhibitors of mushroom tyrosinase. Gene Therapy and Molecular Biology, 16, 201-217.; (**IF_{2013} 0.429; $MEiN_{2014}$ = 15,** according to the new MEiN scores **$MEiN_{2022}$ = 5**).

- Kalathiya, U., **Padariya, M.**, & Baginski, M. (2014). Molecular modeling and evaluation of novel dibenzopyrrole derivatives as telomerase inhibitors and potential drug for cancer therapy. IEEE/ACM Transactions on Computational Biology and Bioinformatics, 11(6), 1196-1207. doi:10.1109/tcbb.2014.2326860; (**IF_{2014} 1.438; $MEiN_{2014}$ = 25,** according to the new MEiN scores **$MEiN_{2022}$ = 70**).

- Kalathiya, U., & **Padariya, M.** (2014). Inhibiting activity of HIV-1: protease, reverse transcriptase and integrase all together by novel compounds using computational

approaches. International Journal of Bioscience, Biochemistry and Bioinformatics, 4(6), 448-457. doi:10.17706/ijbbb.2014.4.6.448-457

- Kalathiya, U., **Padariya, M.**, Jewginski, M., & Baginski, M. (2014). Molecular docking studies towards development of novel Gly-Phe analogs for potential inhibition of Cathepsin C (dipeptidyl peptidase I). International Journal for Computational Biology, 3(1), 3-26.

- **Padariya, M.**, Kalathiya, U., & Georrge, J. (2015). Easy access tool for small interfering RNA (siRNA) data. In proceedings of 8th National Level Science Symposium, Christ College, 2, 129-133. (ISBN: 9788192952116, Christ Publications, accessed from www.ss.christcollegerajkot.edu.in).

In the period June-September 2015, I did my scientific internship in Prof B. Jayaram lab., at The Supercomputing Facility for Bioinformatics & Computational Biology (SCFBio), Indian Institute of Technology (IIT), New Delhi, India (www.scfbio-iitd.res.in). During my internship I worked on the project entitled: "Designing new compounds based on dibenzopyrrole structure to improve its efficiency as telomerase inhibitors applying fragment-based approaches". In the laboratory of Prof. B. Jayaram at the Indian Institute of Technology (Delhi, India), I gained experience of several techniques that can be used for protein structure prediction, drug designing, and docking purposes. In addition, I have worked on improving or finding new ligands for telomerase and applied a different workflow for finding potential inhibitors for telomerase. This novel workflow was tested and validated on our already published dibenzopyrrole derivatives as telomerase inhibitors.

Along with my main PhD project, I participated as co-investigator in different funded projects in the group; (i) New compounds with anticancer activity that disrupt telomere functions. The National Centre for Research and Development, Poland (TARGETTELO; 2017-2019), and (ii) New inhibitors of the catalytic subunit of telomerase. OPUS, National Science Center, Poland (2015-2016). In these projects, I investigated the potential human telomerase mutations that were identified by a systematic computational approach. Moreover, molecular docking methods were used to predict the effects of these mutations on the affinity of certain ligands. In addition, applying structure-based virtual screening and fragment-based approach I designed new parent molecules and its derivatives against the TRF2 protein from the shelterin complex. Considering crucial findings of the project, we have filed a patent application in which we identified novel inhibitors of interactions between TRF1-TIN2 or TRF2-TIN2 telomeric proteins for use in anticancer therapy.

- PZ/8885/RW/PCT, Inhibitors of interactions between TRF1-TIN2 or TRF2-TIN2 telomeric proteins for use in anticancer therapy. Visegrad Patent Institute (ISA/XV), 2022 (patent application).

Established collaborations

Upon completion and receiving my doctoral degree in November 2018, I joined University of Gdansk as adiunkt / postdoctoral researcher at International Centre for Cancer Vaccine Science (ICCVS), which gave me an opportunity to participate in several projects ongoing in the group. Collaborating with different researchers from distinct academic universities resulted in 17 papers (after doctoral degree) and participation in several grant applications. In addition

to my 8 papers included as scientific achievements, other 9 published papers after obtaining doctoral degree were:

- **Padariya, M.**, Kalathiya, U., Houston, D. R., & Alfaro, J. A. (2020). Recognition dynamics of cancer mutations on the ERp57-Tapasin interface. Cancers, 12(3), 737. https://doi.org/10.3390/cancers12030737 **(IF_{2020} 6.64; $MEiN_{2020}$ = 140)**.

- Brankiewicz, W., Kalathiya, U., **Padariya, M.,** Węgrzyn, K., Prusinowski, M., Zebrowska, J., Zylicz-Stachula, A., Skowron, P., Drab, M., Szajewski, M., Ciesielski, M., Gawrońska, M., Kallingal, A., Makowski, M., & Bagiński, M. (2023). Modified peptide molecules as potential modulators of shelterin protein functions; TRF1. Chemistry (Weinheim an der Bergstrasse, Germany), e202300970. Advance online publication. https://doi.org/10.1002/chem.202300970 **(IF_{2022} 4.30; $MEiN_{2020}$ = 140)**.

- Singh, A., **Padariya, M.,** Faktor, J., Kote, S., Mikac, S., Dziadosz, A., Lam, T. W., Brydon, J., Wear, M. A., Ball, K. L., Hupp, T., Sznarkowska, A., Vojtesek, B., & Kalathiya, U. (2022). Identification of novel interferon responsive protein partners of human leukocyte antigen A (HLA-A) using cross-linking mass spectrometry (CLMS) approach. Scientific reports, 12(1), 19422; https://doi.org/10.1038/s41598-022-21393-z **(IF_{2021} 4.996; $MEiN_{2021}$ = 140)**.

- Mikac, S., Dziadosz, A., **Padariya, M.,** Kalathiya, U., Fahraeus, R., Marek-Trzonkowska, N., Chruściel, E., Urban-Wójciuk, Z., Papak, I., Arcimowicz, L., Marjanski, T., Rzyman, W., Sznarkowska, A. Keap1-resistant ΔN-Nrf2 isoform does not translocate to the nucleus upon electrophilic stress. bioRxiv 2022.06.10.495609; doi: https://doi.org/10.1101/2022.06.10.495609.

- Kalathiya, U., **Padariya, M.,** Faktor, J., Coyaud, E., Alfaro, J. A., Fahraeus, R., Hupp, T. R., & Goodlett, D. R. (2021). Interfaces with structure dynamics of the workhorses from cells revealed through cross-linking mass spectrometry (CLMS). Biomolecules, 11(3), 382. https://doi.org/10.3390/biom11030382 **(IF_{2020} 4.88; $MEiN_{2021}$ = 100)**.

- Kalathiya, U., **Padariya, M.,** Fahraeus, R., Chakraborti, S., & Hupp, T. R. (2021). Multivalent display of SARS-CoV-2 Spike (RBD domain) of COVID-19 to nanomaterial, protein ferritin nanocages. Biomolecules, 11(2), 297. https://doi.org/10.3390/biom11020297 **(IF_{2020} 4.88; $MEiN_{2021}$ = 100)**.

- Kalathiya, U., **Padariya, M.,** Mayordomo, M., Lisowska, M., Nicholson, J., Singh, A., Baginski, M., Fahraeus, R., Carragher, N., Ball, K., Haas, J., Daniels, A., Hupp, T. R., & Alfaro, J. A. (2020). Highly conserved homotrimer cavity formed by the SARS-CoV-2 Spike glycoprotein: a novel binding site. Journal of clinical medicine, 9(5), 1473. https://doi.org/10.3390/jcm9051473 **(IF_{2020} 4.24; $MEiN_{2020}$ = 140)**.

- Kalathiya, U., **Padariya, M.**, Pawlicka, K., Verma, C. S., Houston, D., Hupp, T. R., & Alfaro, J. A. (2019). Insights into the effects of cancer associated mutations at the UPF2 and ATP-binding sites of NMD master regulator: UPF1. International journal of molecular sciences, 20(22), 5644. doi:10.3390/ijms20225644 **(IF_{2019} 4.556; $MEiN_{2019}$ = 140)**.

- Kalathiya, U., **Padariya, M.**, & Baginski, M. (2019) Structural, functional, and stability change predictions in human telomerase upon specific point mutations, Scientific Reports, 9, 8707. doi:10.1038/s41598-019-45206-y (**IF_{2019} 3.998; $MEiN_{2019}$ = 140**).

Moreover, I had an privilege and opportunity to work with several well recognized scientists in the field, which helped me to establish collaborations with several national and international researcher and they were:

- dr. Konstantinos Karakostis, Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain - molecular biology and evolution.

- prof. Fritz Vollrath, Department of Zoology, Zoology Research and Administration Building, University of Oxford, Oxford, UK - molecular biology and evolution.

- Alison Daniels, Department of Infectious Disease, Edinburgh, Scotland EH4 2XR, UK – validation of drugs in SARS-CoV-2 infected cells.

- dr. Christine Tait-Burkard, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom – validation of drugs in SARS-CoV-2 infected cells.

- prof. Borek Vojtesek, from RECAMO, Masaryk Memorial Cancer Institute, Zlutykopec 7, 65653 Brno, Czech Republic – proteomics and mass spectrometry approach to trace proteins.

- dr. Minofar Babak, from the Faculty of Science, University of South Bohemia, Branišovská 1760, 37005 Česk é Budějovice, Czech Republic – ionic liquids and structural biology.

- dr. Soumyananda Chakraborti, National Institute of Malaria Research, Dwarka, New Delhi 110077, India – ferritin nanoparticles and its implications.

Summary of scientific achievement

My PhD (dr inż.) degree was awarded from Faculty of Chemistry, Gdańsk University of Technology and Master in engineering (mgr inż.) from Faculty of Chemistry, Wrocław University of Science and Technology. Poland. In addition, I received scientific training from Indian Institute of Technology (IIT), New Delhi, India in the group of Prof B. Jayaram at The Supercomputing Facility for Bioinformatics and Computational Biology department. I presented my scientific data in several conference proceedings which included 3 oral and 5 poster presentations. During my doctoral studies, I was awarded for three consecutive years the best PhD students from Gdańsk University of Technology, and my PhD was funded by the Polish National Agency for Academic Exchange (NAWA). In the year 2015 and 2017, I received an award for the "Young scientists with best creative work published" by Polish Academy of Sciences (PAN), Gdansk. My scientific achievements include a total of 33 articles published in scientific journals, and 1 patent application. Among these articles, 28 articles were published in the JCR listed journal and 5 articles in national / international journals. Additionally, 8 scientific articles presented for evaluation demonstrate the pharmacophore

models for diverse sets of proteins or enzymes involved in cancer or immune response. I am the first author in 17 of the articles published, and the total impact factor of all my publications is 115.602, which have a total 240 citations (as of Scopus database and accessed on 22 August 2023). The total score as per the list of the Ministry of Education and Science (Ministerstwo Edukacji i Nauki; MEiN) is 2640 points. Of my 33 articles, 10 publications consist of 100 MEiN points, 7 publications for 140 MEiN points, and 1 publication for 200 MEiN points.

Along with participating in several grant applications, I have been actively involved in participation in funded projects which includes (i) Quantifying binding and interaction dynamics of peptide-loading complex components upon viral interference, UGrants-START (grant agreement no. 1220.6010.24.2022), 2022 (I was the principal investigator). (ii) Co-investigator: "Specificity in detection of PTCs in mRNA by NMD and its network, insights from cancer perspective and cross-linking (XL-MS). SONATINA, National Science Center, Poland (grant agreement no. 2020/36/C/NZ2/00108), 2020-2023". (iii) Co-investigator; "The impact of UPF1 ATP mimetics on the mutant immunopeptidome. OPUS, National Science Center, Poland (grant agreement no. 2020/39/B/NZ7/02677), 2021-2025". (iv) Co-investigator, "New compounds with anticancer activity that disrupt telomere functions. The National Centre for Research and Development, Poland (TARGETTELO; grant agreement no. STRATEGMED3/306853/9/NCBR/2017), 2017-2018". (v) Co-investigator: "New inhibitors of catalytic subunit of telomerase. OPUS, National Science Center, Poland (grant agreement no. 2014/13/B/NZ7/02207), 2015-2016. (vi) Co-investigator, "Characterisation of the Function and Regulation of Nrf2 Isoform 2". SONATA, National Science Center, Poland (grant agreement no. 2021/43/D/NZ1/02059), 2022-2025". As a contribution in the development of the scientific community, I have reviewed several manuscripts in different JCR listed journals. Additionally, I am serving as guest editor of the special Issue "SARS-CoV-2 Spike-Based Vaccines" in Vaccines journal.

- **Total number of publications: 33** (before doctoral degree: **16**, and after doctoral degree: **17**).
- **Scientific achievement: 8 papers** (first author in all papers). **IF = 56.524,** MEiN = **910.**
- **The total number of publications excluding scientific achievements: 25** (first corresponding or senior corresponding author **9** papers). **IF = 59.078,** MEiN = **1730.**
  - before doctoral degree: **16** papers. **IF = 20.588,** MEiN = **690.**
  - after doctoral degree: **9** papers. **IF = 38.49,** MEiN = **1040.**

- **The overall impact factor journals**, by year of publication: **115.602** (papers before doctoral degree: IF = 20.588, papers after doctoral degree: IF = **95.014**).
- **The total number of MEiN points**: **2640** (papers before doctoral degree: MEiN = **690,** papers after doctoral degree: MEiN = **1950**).
- **Number of citations** based on databases accessed on 22 August 2023): **210** (Web of Science), **240** (Scopus), and **313** (Google Scholar).
- **Hirsch's index** according to Web of Science **H = 9**, Scopus **H = 8**, and Google Scholar **H = 10** (accessed on 22 August 2023).

- Number of **participations in funded projects: 6.**

- Conference presentations: 3 oral and 5 poster presentations.

Supplementary Literatures

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6. Presentation of teaching and organizational achievements as well as achievements in popularization of science or art

Teaching achievements and contributions regarding dissemination of science

Computer Science laboratories at Department of Physical Chemistry, Faculty of Chemistry, Gdansk University of Technology (Environmental Protection course in English) (2014-2016).

Scientific supervision of students

I am a co-advisor of a PhD student from International Centre for Cancer Vaccine Science, University of Gdańsk.

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.**

I received the young scientists award for the best creative work published in 2015 and 2017, by PAN (Polska Akademia Nauk) Gdańsk, Poland for the works; "Padariya, M., Kalathiya, U., & Baginski, M. (2018). Structural and dynamic insights on the EmrE protein with TPP^+ and related substrates through molecular dynamics simulations. Chemistry and Physics of Lipids, 212, 1-11" and "Padariya, M., Kalathiya, U., & Baginski, M. (2015). Structural and dynamic changes adopted by EmrE, multidrug transporter protein-studies by molecular dynamics simulation. Biochimica Et Biophysica Acta (BBA) - Biomembranes, 1848(10), 2065-2074", respectively. In addition, during my doctoral studies I was a member of the EUROPIN PhD Program (2014-2018; https://www.univie.ac.at/europin). I have received the III-degree award of rector from University of Gdansk, for my scientific work in 2021.

Moreover, I received the Ignacy Łukasiewicz Scholarship funded by the Polish National Agency for Academic Exchange (NAWA), Poland during my doctoral studies. I was awarded scholarships such as Pro-quality (for scientific achievements from Faculty of Chemistry), best PhD students (from Rector of Gdańsk University of Technology) and InterPhD scholarship for scientific achievements from Gdańsk University of Technology, Gdańsk, Poland.

A detailed list of all my scientific achievements can be found in the Appendix 4.

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(Applicant's signature)

Monikaben Padariya