List of scientific or artistic achievements which present a major contribution of dr. Łukasz Pepłowski to the development of a specific discipline

I. INFORMATION ON SCIENTIFIC OR ARTISTIC ACHIEVEMENTS SET OUT IN ART. 219 PARA 1. POINT 2 OF THE ACT

1. Cycle of scientific articles related thematically, pursuant to art. 219 para 1. point 2b of the Act;

The articles listed below are placed according to the order of appearance in the „Summary of Professional Accomplishments". Corresponding authors are marked with an asterisk. If several authors had equal "first-author" contributions, they are marked with a cross. The parameter of the journal's impact factor (IF, "Impact factor") is given based on data from 2021 (published in 2022). The Ministry's points are taken from the list published in the announcement of December 21, 2021.

1.1. [**H1**] *Insight into the broadened substrate scope of nitrile hydratase by static and dynamic structure analysis*

Dong Ma†, Zhongyi Cheng†, **Łukasz Pepłowski**†, Laichuang Han, Yuanyuan Xia, Xiaodong Hou, Junling Guo, Dejing Yin, Yijian Rao*, Zhemin Zhou*

Chemical Science 13(28) 8417-8428 (2022);

Impact Factor: 9.969;

Ministry's points: 200;

Number of citations: 0 (GS); 0 (WoS);

The work is devoted to broadening the spectrum of nitriles catalyzed by the key biotechnological enzyme nitrile hydratase (NHase) and increasing the catalytic activity of this enzyme. In this work, two mutant variants of NHase from the organism *Pseudonocardia Thermophila* JCM 3095 were studied experimentally and theoretically. The newly designed and created variants, βM46R and βA129R, proved to be significantly more efficient than the native protein. Significant improvements were made in obtaining enzymatic reactions for very large nitriles as substrates. For both mutational variants, crystals were obtained and the 3D structure deposited in the PDB database (pdb codes 7W8L and 7W8M) was solved. Molecular dynamics simulations performed on the crystallographic structures explained the reasons for success, i.e., increased catalytic activity of both variants.

My contribution to the work:

- Analysis of native NHase with pdb code 1IRE to determine amino acids for modification
- Participation in the solving of the crystallographic structure by validating the data obtained from the experiment and comparing them with previously published data
- Development and implementation of a dedicated molecular dynamics (MD) computational protocol
- Preparation of inputs for calculations
- Performing and analyzing all MD simulations
- Comparison of MD data with experimental data (together with collaborators from China)
- Elucidating, based on the MD simulations performed, the reason for the increased catalytic activity of NHase
- Writing those parts of the article that relate to the research with theoretical methods

1.2. [**H2**] *"Toolbox" construction of an extremophilic nitrile hydratase from Streptomyces thermoautotrophicus for the promising industrial production of various amides*

Junling Guo, Julia Berdychowska, Qianpeng Lai, Yiwei Meng, Zhongyi Cheng*, **Łukasz Pepłowski***, Zhemin Zhou*

International Journal of Biological Macromolecules 221 1103-1111 (2022);

Impact Factor: 8.025;

Ministry's points: 100;

Number of citations: 0 (GS); 0 (WoS);

This study investigated the reasons for the enhanced catalytic activity of NHase from the organism *Streptomyces thermoautotrophicus* (*St*NHase) in the βL48D mutational variant. The newly developed enzyme shows significantly better catalytic activity towards nicotinonitrile than the native one. The created homology model of *St*NHase allowed to identify several amino acids for modification. Analysis by experimental biotechnology methods of mutational variants at positions determined from the model allowed to select the most active one, i.e. βL48D. Using MD, the differences in the structure of the WT protein and βL48D were determined, and the physical reasons for the increased catalytic activity of the new variant were elucidated.

My contribution to the work:

- Supervise and help a PhD student in developing a homology model of *St*NHase
- Proposing and implementation of a molecular dynamics computational protocol

- Supervision of the accuracy of the MD calculations
- Joint analysis with a PhD student of the results obtained by computational biophysics methods i.e. MD trajectories
- Using CAVER program to analyze static structures and MD simulations.
- Selecting amino acids for modification.
- Elucidation, based on the MD simulations performed, of the physical reasons for the increased catalytic activity of this form of NHase
- Writing the parts of the article that deal with the research conducted by theoretical methods

1.3. [**H3**] *Improving the thermostability and catalytic efficiency of the subunit-fused nitrile hydratase by semi-rational engineering*

Yuanyuan Xia, **Łukasz Pepłowski**, Zhongyi Cheng, Tianyi Wang, Zhongmei Liu, Wenjing Cui, Michihiko Kobayashi*, Zhemin Zhou*

ChemCatChem 10(6) 1370-1375 (2018);

Impact Factor: 5.497;

Ministry's points: 100;

Number of citations: 21 (GS); 19 (WoS);

The work aimed to design and synthesize more thermostable (relative to the WT protein) variants of NHase with the β and α amino acid chains of NHase fused into a single monomeric system. Using bioinformatics tools and theoretical computational biophysics methods, a series of mutational variants of this enzyme were suggested, and their thermostability and catalytic activity were experimentally tested. The best three variants showing significantly better thermostability and slightly increased catalytic activity were examined by MD to explain the increased thermostability of these variants. It turned out that all three mutational variants induce the formation of new hydrogen bonds in the protein, which positively affects thermostability.

My contribution to the work:

- Preparation of the Fus-NHase homology model
- Development and implementation of a molecular dynamics (MD) computational protocol
- Preparation of inputs for calculations
- Performing and analyzing all MD simulations
- Identification of possible promising mutational variants
- Writing those parts of the article that directly relate to the study conducted by theoretical methods

1.4. [**H4**] *Computational design of nitrile hydratase from Pseudonocardia thermophila JCM3095 for improved thermostability*

Zhongyi Cheng, Yao Lan, Junling Guo, Dong Ma, Shijin Jiang, Qianpeng Lai, Zhemin Zhou*, **Łukasz Pepłowski***

Molecules 25(20) 1-18 (2020);

Impact Factor: 4.927;

Ministry's points: 140;

Number of citations: 14 (GS); 12 (WoS);

The aim of this study was to design a more thermostable variant of NHase derived from the organism *Pseudonocardia thermophila* JCM3095 using bioinformatics tools and to elucidate the reasons for the increased thermostability. FireProt server was selected as the design tool. The experimental analysis confirmed that the proposed variant, containing 10 mutations, has significantly increased thermostability compared to the native protein and increased catalytic activity against nicotinonitrile. Analysis of the MD trajectories allowed us to clarify in detail the effect of each introduced mutation on thermostability.

My contribution to the work:

- Establishing the concept of the study
- Determination of mutations using bioinformatics tools
- Development and implementation of the MD calculation protocol
- Preparation of inputs for calculations
- Performing and analyzing the results of all MD simulations
- Explanation, based on the MD simulations, of the influence of each mutation on thermostability enhancement.
- Writing the article (in addition to the experimental data)

1.5. [**H5**] *Effect and mechanism analysis of different linkers on efficient catalysis of subunit-fused nitrile hydratase*

Junling Guo, Zhongyi Cheng, Julia Berdychowska, Xiaonan Zhu, Lingling Wang, **Łukasz Pepłowski***, Zhemin Zhou*

International Journal of Biological Macromolecules 181 444-451 (2021);

Impact Factor: 8.025;

Ministry's points: 100;

Number of citations: 11 (GS); 8 (WoS);

The work aimed to show that the introduction of protein linkers between the two β and α chains of the NHase enzyme results in increased thermostability. In the

study, tetrameric NHase from the organism *Pseudomonas putida* NRRL-18668 (*Pp*NHase) was modified by introducing three types of linkers that differed in a protein sequence. Each type of linker was further characterized by a different number of linker sequence repeats. All the experimentally obtained NHases showed to be more thermostable than the WT protein. The most thermostable variant was the one in which eight repeats of the helical linker composed of EAAAK amino acids were introduced. Analysis of the static structures and the results of molecular dynamics simulations made it possible to clarify the physicochemical reasons for the increased thermostability of the modified NHases.

My contribution to the work:

- Development of homology models of three NHase variants with different linker types.
- Development and implementation of a computational protocol for molecular dynamics simulations
- Preparation of inputs for calculations
- Performing all MD simulations
- Analysis (together with Julia Berdychowska, M.Sc.) of all MD simulations
- Clarification of the reasons for the increased thermostability.
- Writing parts of the article directly related to the research using theoretical methods

1.6. [**H6**] *Development of thermostable sucrose phosphorylase by semi-rational design for efficient biosynthesis of alpha-D-glucosylglycerol*

Yuanyuan Xia, Xiaoyu Li, Linli Yang, Xiaozhou Luo, Wei Shen, Yu Cao, **Łukasz Pepłowski***, Xianzhong Chen*

Applied Microbiology and Biotechnology 105(19) 7309-7319 (2021);

Impact Factor: 5.56;

Ministry's points:100;

Number of citations: 9 (GS); 6 (WoS);

This study investigated the possibility of increasing the thermostability of the enzyme sucrose phosphorylase (SPase). Several mutational variants were proposed using bioinformatics tools and computational theoretical biophysics methods to have increased thermostability. Experimental studies confirmed the increased thermostability (and slightly catalytic activity) of the four SPase variants. It was noted that the variant containing all four mutations showed slightly worse thermostability than the best T219L mutant. MD simulations of the models helped to explain why the T219L variant and the one containing all four mutations are more thermostable than the native variant. In the publication, we rationally explained why the variant containing 4 mutations is not as thermostable as the T219L variant.

My contribution to the work:

- Development of the SPase homology model
- Conceptualization and implementation of a molecular dynamics (MD) computational protocol
- Preparation of inputs for calculations
- Performing all MD simulations
- Bioinformatics analysis and identification of mutational variants to be analyzed experimentally.
- Analysis of MD simulation results.
- Elucidation of structural reasons for increased thermostability of new SPase variants.
- Writing the parts of the article on the research with theoretical methods

1.7. [**H7**] *Identification of key residues modulating the stereoselectivity of nitrile hydratase toward rac-mandelonitrile by semi-rational engineering*

Zhongyi Cheng, **Łukasz Pepłowski**, Wenjing Cui, Yuanyuan Xia, Zhongmei Liu, Jialei Zhang, Michihiko Kobayashi, Zhemin Zhou*

Biotechnology and Bioengineering 115(3) 524-535 (2018);

Impact Factor: 4.395;

Ministry's points: 100;

Number of citations: 26 (GS); 21 (WoS);

The aim of the work was to explain how to design an enzyme featuring stereoselectivity and to explain the reasons for this stereoselectivity. The NHase from the organism *Rhodococcus rhodochrous* J1, which naturally catalyzed both enantiomers of the mandelonitrile racemate, was studied. Using docking simulations and steered molecular dynamics (SMD) applied to a homology model of the native variant of the enzyme, amino acids that may affect the selective function of the enzyme were determined. All mutational variants of the theoretically determined amino acids were experimentally checked. It was found that the βF37H variant catalyzes mainly S-mandelonitrile and hardly catalyzes R-mandelonitrile. Using docking methods and SMD simulations performed in addition for the βF37H variant, the reasons for the stereoselectivity in the newly designed variant were clarified.
My contribution to the work:

- Preparation of NHase homology model
- Development and implementation of a computational protocol for the realization of molecular dynamics, steered molecular dynamics and docking simulations
- Preparation of inputs for calculations

- Performing all MD, SMD and docking calculations
- Analysis of MD, SMD and docking simulations
- Clarification of the reason for the increased stereoselectivity of the βF37H variant
- Writing those parts of the article that directly relate to the study by theoretical methods

1.8. [**H8**] *Modulation of nitrile hydratase regioselectivity towards dinitriles by tailoring the substrate binding pocket residues*

Zhongyi Cheng, Wenjing Cui, Yuanyuan Xia, **Łukasz Pepłowski**, Michihiko Kobayashi*, Zhemin Zhou*

ChemCatChem 10(2) 449-458 (2018);

Impact Factor: 5.497;

Ministry's points: 100;

Number of citations: 14 (GS); 11 (WoS);

The work aims to explain how to design enzymes featuring regioselectivity. Here we studied how to modify NHase from the organism *Rhodococcus rhodochrous* J1 to catalyze dinitriles (aliphatic and aromatic) selectively, i.e. in such a way that only one of the nitrile groups is hydrated (which is practically impossible using classical methods of chemical technology). The homology model was developed and the results of docking of four different dinitriles to the model made it possible to determine the amino acids important in orienting the substrates toward the catalytic center. Modifying these amino acids by saturated mutagenesis made it possible to find two variants having a regioselectivity reaching 97% (i.e. 97% of the catalysis product had one nitrile group and one amide group, and 3% were diamides). Making both mutations at once allowed us to develop an enzyme that catalyzed two of the four tested dinitriles to 100% to nitrilamide, and the other two to at least 97% to nitrilamide. Docking dinitriles and nitrilamides to both NHase variants helped elucidate the reasons for the increased regioselectivity of the modified NHase.

My contribution to the work:

- Preparation of the NHase homology model
- Development and implementation of a computational protocol for molecular dynamics and docking simulations
- Preparation of inputs for calculations
- Performing all MD and docking calculations
- Analysis of MD and docking simulation results
- Elucidation of the reason for the increased regioselectivity of the newly developed NHase variants
- Writing parts of the article related to the research by theoretical methods

II. INFORMATION ON SCIENTIFIC OR ARTISTIC ACTIVITY

1. List of articles published in scientific journals (including the articles not mentioned in section I.2).

The articles published after the doctoral degree are listed below in chronological order. Corresponding authors are marked with an asterisk. If several authors had equal "first-author" contributions, they were marked with a cross. The IF (Impact Factor) value is given based on data from 2021 (published in 2022). Ministry points are from the list published in the communication of December 21, 2021.

- [P1] *Molecular jamming: the cystine slipknot mechanical clamp in all-atom simulations*

  Łukasz Pepłowski, Mateusz Sikora, Wiesław Nowak, Marek Cieplak*

  Journal of Chemical Physics 134(8) 085102:1-14 (2011);

  Impact Factor: 4.304.;

  Ministry's points: 100;

  Number of citations: 21 (GS); 12 (WoS);

  In this work, a group of 20 proteins identified in earlier work conducted by Prof. Marek Cieplak as those that are most difficult to unfold using simplified "Go-like" models, in which each amino acid is defined as a single pseudoatom, were examined using all-atom steered molecular dynamics (SMD), a virtual atomic force microscope. Since Go-like methods do not provide full insight into all molecular processes occurring during simulated protein stretching, all-atom molecular dynamics was used. Two types of proteins were studied: those containing cysteine knots and classical proteins containing no covalent bonds between amino acid side chains. As a comparison, a simulation was also performed for the titin protein, which is the best studied protein in terms of the stretching scenario. Using SMD simulations, we explained why certain proteins containing cysteine knots have a high stretching force (the presence of large aromatic amino acids that need to be "threaded" through the cysteine-containing ring) and others require less force. It was also explained that some classical proteins need a high force to stretch because they form many hydrogen bonds between two long β-strands, which need to be broken synchronously.

  My contribution to the paper:

  - Development and implementation of a computational protocol and preparation of inputs for calculations
  - Performing all-atom models of 21 proteins and performing a series of all-atom steered molecular dynamics simulations for them
  - Writing a tool in Perl language for analysis of SMD results, to determine (among other things): evolution of applied force, stretching length, work

- Analysis of all SMD simulations (determination of stretching forces, explanation of molecular reasons for different stretching scenarios)
- Analysis of the change in maximum stretching force as a function of stretching speed
- Article editing

- [P2] *Nanomechanics of Ig-like domains of human contactin (BIG-2)*

  Karolina Mikulska, **Łukasz Pepłowski**, Wiesław Nowak

  Journal of Molecular Modeling 17(9) 2313-2323 (2011);

  Impact Factor: 2.172;

  Ministry's points: 40;

  Number of citations: 10 (GS); 8 (WoS);

  In this study, a modular inter-synaptic gap protein: contactin, was investigated using steered molecular dynamics (SMD). This protein contains six IgC2-type domains and four FnIII-type domains with a different architectures. Using SMD simulations, we determined the stretching scenarios of each IgC2-type domain, which allowed us to better understand the atomic force microscopy (AFM) stretching experiments of single proteins performed earlier.

  My contribution to the work:

  - Providing a program written in Perl to analyze SMD simulations
  - Assistance in setting up the computational protocol.
  - Assistance in the analysis of SMD simulations

- [P3] *Statistical properties of spectra of chloronaphthalenes*

  Dorota Bielińska-Wąż, Piotr Wąż, Timothy Clark, Tomasz Puzyn,

  Łukasz Pepłowski, Wiesław Nowak

  Journal of Mathematical Chemistry 51(3) 857-867 (2013);

  Impact Factor: 2.413;

  Ministry's points: 70;

  Number of citations: 4 (GS); 4 (WoS);

  The work aimed to show that with the help of "spectral density distribution moments" used as molecular descriptors, it is possible to correctly distinguish different molecules based on a statistical analysis of their spectra and also to check whether the quantum calculations carried out are incorrect. In this paper, the infrared spectra of a series of 76 chloronaphthalenes were determined using quantum chemistry methods (DFT/B3LYP 6-311++G**). The spectra were then analyzed statistically by determining the spectral density distribution moments. It turned out that these moments carry important information about the molecular structure of the studied compounds.

My contribution to the work:

- Performing quantum chemical calculations for 76 chloronaphthalenes by DFT method
- Determination and analysis of infrared spectra of chloronaphthalenes based on quantum calculations

- [P4] *Mechanical transition in a highly stretched and torsionally constrained DNA*

  Janusz Strzelecki*, **Łukasz Pepłowski**, Robert Lenartowski, Wiesław Nowak,

  Aleksander Balter

  Physical Review E 89 02701:1-15 (2014);

  Impact Factor: 2.707;

  Ministry's points: 140;

  Number of citations: 8 (GS); 6 (WoS);

  The purpose of this study was to elucidate the formation of a previously unknown plateau of forces during the stretching and folding of single DNA molecules using AFM. During repeated stretching of the same DNA molecule, in rare cases, a clear difference can be observed in the force applied to the AFM microscope tip during stretching and folding. It has been observed that this happens when a double strand of DNA firmly "sticks" to the AFM tip with both strands, preventing the DNA double helix from rotating. The unusual plateau was not observed when only one strand was attached to the tip. In an effort to explain these differences in DNA nanomechanics, steered molecular dynamics calculations of the DNA double strand were performed in two cases: with the ability of rotation along the axis of the double strand and with restraints applied to prevent rotation. It turned out that in both cases the final DNA structure is different. The initial structure of B-DNA changes to that of Zip-DNA in simulations without constraints and P-DNA in simulations with constraints. All of the above structures have different lengths at a given number of nucleotide base pairs. The transition lengths from the B-DNA to the P-DNA form, determined with SMD, were in good correspondence with the point of formation of the force plateau, which allowed us to elucidate its formation mechanism at the molecular level.

  My contribution to the work:

  - Creation of DNA models
  - Development and implementation of a new computational protocol that allows to disable DNA rotation during stretching in SMD
  - Preparation of inputs for calculations
  - Implementation of calculations and analysis of SMD simulations

- [P5] *Metallochaperone function of the self-subunit swapping chaperone involved in the maturation of subunit-fused cobalt-type nitrile hydratase*

  Yuanyuan Xia, **Łukasz Pepłowski***, Zhongyi Cheng, Tianyi Wang, Zhongmei Liu, Wenjing Cui, Michihiko Kobayashi, Zhemin Zhou*

  Biotechnology and Bioengineering 116(3) 481-489 (2019);

  Impact Factor: 4.395;

  Ministry's points: 100;

  Number of citations: 10 (GS); 9 (WoS);

  An active nitrile hydratase (NHase) requires the presence of a non-standard active site containing an iron ion or cobalt ion. Currently, only cobalt hydratases are used in the industrial, biotechnological conversion of nitriles to amides. Activation of the enzyme is carried out by chaperone proteins called NHase activators, which deliver the metal ion to the catalytic center. In the paper [P5], two Co-NHase activators from two different organisms were studied. The activators were named P14K and NhlE. Despite the slight sequence similarity and a different number of amino acids, the two proteins show similar activation properties. The work aimed to understand the enzyme nitrile hydratase's activation process and the structure of the two activators better. It was shown that the amino acids located at the C-terminus of the activators have a key role in the activation process. Using molecular modeling tools (de novo folding), 3D models of the proteins were built. It was shown that key amino acids for activation can form structures that encourage cobalt ion binding.

  My contribution to the work:

  - Development based on the amino acid sequence of two 3D models of NHase activators, using the QUARK tool (de novo folding)
  - Comparison of the structures of the two models
  - Conceptualization and implementation of a computational protocol and preparation of inputs for calculations
  - Performing simulations by molecular dynamics methods and showing that the fragments responsible for binding the cobalt ion exhibit incredibly high mobility
  - By using the simulated annealing method, I showed that amino acids essential in NHase activation can form structures that promote the binding of the cobalt ion

- [P6] *Enhancing thermostability and activity of sucrose phosphorylase for high-level production of 2-O-α-d-glucosylglycerol*

  Linli Yang, **Łukasz Pepłowski**, Yujuan Shen, Haiquan Yang, Xianzhong Chen, Wei Shen*, Yuanyuan Xia*

  Systems Microbiology and Biomanufacturing (Springer) **2** 643-652 (2022);

  Impact Factor: None (newly established journal).;

  Ministry's points: 0;

  Number of citations: 1 (GS); 0 (WoS);

  The work aimed to design a biotechnological enzyme sucrose phosphorylase (SPase) exhibiting enhanced thermostability together with increased catalytic activity, and to elucidate the reasons for the improved properties after appropriate mutations. The studied native enzyme was the same as in the work [**H6**]. More stable and active variants were designed using the PROSS server. In this work, the catalytic activity and thermostability of 4 suggested mutational variants of SPase were experimentally verified. Mutations of the two variants showing the highest activity were combined in a single protein, obtaining an enzyme with almost doubled activity than the native variant. Since thermostability (high reaction temperature prevents the growth of unwanted microorganisms) is more important in a biotechnological process using SPase than high catalytic activity, the molecular reasons for the increased thermostability of the V23L variant in comparison to the native enzyme were explained using MD simulations.

  My contribution to the work:

  - Development of a homology model of SPase from the organism *Leuconostoc mesenteroides* ATCC 12291 and its V23L variant
  - Conceptualization and implementation of the computational protocol and preparation of inputs for calculations
  - Running molecular dynamics simulations of both variants at standard (300K) and increased (335K) temperatures
  - Analyzing the results of molecular dynamics simulations and clarifying the reason for the increased thermostability of the V23L variant. It turned out that leucine forms a more stable hydrophobic core than valine, and thus the thermostability is increased
  - Writing these sections of the article directly related to the study of theoretical methods

  The manuscript, despite its similar subject to the series of papers presented as a postdoctoral achievement, is not included in the main achievement because it was published in a newly established scientific journal published by the Springer publishing house. This journal does not yet have a designated "Impact Factor" or ministerial points.

- [P7] *Vibrational spectroscopy studies of methacrylic polymers containing heterocyclic azo dyes*

  Łukasz Pepłowski*, Robert Szczęsny, Łukasz Skowroński, Anastasiia Krupka, Vitaliy Smokal, Beata Derkowska-Zielińska*

  Vibrational Spectroscopy 120 1-8 (2022);

  Impact Factor: 2.382;

  Ministry's points: 40;

  Number of citations: 0 (GS); 0 (WoS);

  In this paper, an infrared analysis of a group of polymerized azo dyes was performed. These compounds exhibit photo chromaticity. The studied group of compounds (4 compounds) differed by a substituent in the para position in one of the aromatic rings (in the phenyl group). The series of compounds were examined by Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR) and infrared spectra determined by quantum calculations (DFT/B3LYP 6-311++G(d,p)). Analysis of the spectra showed very high agreement between FTIR and DFT IR spectra. Thanks to quantum chemical calculations, it was possible to describe all maxima of the experimental spectra. PED (Potential Energy Distribution) analysis of the spectra was carried out. Thanks to the conducted research, it will be easy to detect the studied compounds by infrared spectroscopy.
  My contribution to the work:

  - Carrying out quantum chemical calculations for a group of dyes with an azobenzene group (each with about 65 atoms)
  - Analysis of infrared spectra obtained by theoretical methods, together with the assignment of each frequency obtained to the corresponding mode (about 170 modes for each molecule)
  - Comparison of theoretical and experimental results
  - To study the effect of the substituent on the infrared spectrum
  - Examining the effect of the presence of a polymer on the spectrum of a dye
  - PED (Potential Energy Distribution) analysis of spectra obtained by theoretical methods
  - Writing large parts of the article, related to theoretical calculations, and sections on the comparison of the results obtained with DFT IR and FTIR

- [P8] *Bidirectional effect of repeated exposure to extremely low-frequency electromagnetic field (50 Hz) of 1 and 7 mT on oxidative/antioxidative status in rat's brain : the prediction for the vulnerability to diseases*

  Angelika Klimek*, Anna Nowakowska, Hanna Kletkiewicz, Joanna Wyszkowska, Justyna Maliszewska, Milena Jankowska, **Łukasz Pepłowski**, Justyna Rogalska

  Oxidative Medicine and Cellular Longevity 2022 1-14 (2022);

Impact Factor: 7.31

Ministry's points: 100;

Number of citations: 0 (GS); 0 (WoS);

The published results of the study concern the effects of long-term exposure of living organisms (in this case rats) to a magnetic field with a frequency of 50 Hz and an induction of 1mT (weak field for living organisms) and 7mT (strong field for living organisms). In this paper, the effects of exposure on rat behavior, hormone levels and brain structure were studied. It was found that oxidative stress induced by exposure to strong magnetic fields negatively affects hormone levels and prefrontal cortex function. It is suspected that long exposures to magnetic fields may have similar effects on humans.

My contribution to the paper:

- Consultations and discussions on magnetic fields and their interaction with matter in particular animate matter

- [P9] *Tailoring the hinge residue at the substrate access tunnel entrance improves the catalytic performance of industrialized nitrile hydratase toward 3-cyanopyridine*

  Yuanyuan Xia, Meng Yin, **Łukasz Pepłowski***, Zhongyi Cheng*, Zhemin Zhou*

  ChemistrySelect 7(34) 1-6 (2022);

  Impact Factor: 2.307

  Ministry's points: 40;

  Number of citations: 0 (GS); 0 (WoS);

  This work focuses on increasing the catalytic activity of a nitrile hydratase from the organism Rhodococcus rhodochrous J1 (the so-called high molecular weight NHase). Natively, this protein efficiently catalyzes small aliphatic nitriles, but its efficiency is poor against aromatic nitriles (e.g., nicotinonitrile). Using an approach similar to that of the [H2] manuscript, an amino acid at the entrance to the catalytic channel was modified (all possible mutations in amino acid 48 of the β-chain were examined). It turned out that the two variants W48A and W48Y showed very high catalytic activity towards nicotinonitrile (an aromatic nitrile), which increased 5.6 times in the case of the W48Y variant. In addition, it was possible to increase the thermostability of this NHase variant. MD and docking simulations were used to explain the increased activity.

  My contribution to the work:

  - Sharing with the Chinese team the parameters and topologies compatible with the CHARMM 27 force field for a non-standard active site
  - Assistance in establishing the simulation scheme
  - Assistance in the analysis of MD simulation results

- [P10] *Discovery of the ATPase activity of a cobalt-type nitrile hydratase activator and its promoting effect on enzyme maturation*

  Yuanyuan Xia, Zhongyi Cheng, Chen Hou, **Łukasz Pepłowski**, Zhemin Zhou*, Xianzhong Chen*

  Biochemistry (first online DOI:10.1021/acs.biochem.2c00167) 1-8 (2022);

  Impact Factor: 3.321

  Ministry's points: 100;

  Number of citations: 0 (GS); 0 (WoS);

  This work reported for the first time in the scientific literature that the NHase activator NhlE exhibits the properties of an ATPase (meaning it is an enzyme that hydrolyzes ATP to ADP and uses the energy from this hydrolysis). It has been shown that hydrolysis of ATP or GTP is required for proper activation of nitrile hydratase. To explain how ATP binds to the enzyme, molecular modeling methods were used (development of "*de novo*" and homology models of a protein not crystallized so far due to low stability of this protein, molecular dynamics, ligand docking). It was shown that ATP interacts weaker with low-activity activator variants.
  My contribution to the work:
  - Development of "de novo" models in QUARK and C-QUARK tools, as well as the AlphaFold homology model, verifying their quality, comparing them and selecting the best one for further modeling.
  - Assistance in analyzing the results of ATP docking to the WT activator and its mutational variant.

2. Information on presentations given at national or international scientific or arts conferences, including a list of lectures delivered upon invitation and plenary lectures.

The below, from newest to oldest, presents information on conference presentations and lectures. Only postdoctoral entries and only those presented in person are listed.

Conferences:

2022:

- BIT22 Bioinformatics in Torun 2022 – Toruń, Poland (2022-06-23 - 2022-06-25) – **poster;** *Signals Transduction Inside Proteins after Ligand Photoexcitation. Insights from Apomyoglobin Molecular Dynamics Simulation;* Authors: Łukasz Pepłowski, Przemysław Miszta, Wiesław Nowak.
- Biophysics at the Dawn of Exascale Computers; Hamburg, Germany (2022-05-16 - 2022-05-20) – **poster;** *Signals Transduction Inside Proteins after Ligand*

Photoexcitation. Insights from Apomyoglobin Molecular Dynamics Simulation; Authors: Łukasz Pepłowski, Przemysław Miszta, Wiesław Nowak.

- 66th Biophysical Society Annual Meeting; San Francisco, USA (2022-02-19 - 2022-02-23) – **poster;** *Theoretically supported designing of the nitrile hydratase catalytic properties – biotechnologically important metalloenzyme with posttranslational modifications;* Author: Łukasz Pepłowski.

2021:

- BIT21 - BioInformatics in Torun with a session Bioinformatics Fights Viruses; Toruń, Poland (2021-06-24 - 2021-06-24) – **invited lecture;** *Nanomechanical aspects of nanobodies-protein S SARS-CoV-2 virus complexes – a computational study;* Authors: Łukasz Pepłowski, Katarzyna Walczewska-Szewc, Beata Niklas, Julia Berdychowska, Wiesław Nowak.

2019:

- Nano(&)BioMaterials - from theory to applications; Toruń, Poland (2019-06-06 - 2019-06-07) - **lecture;** *Transport of drugs through the cell membrane by carbon nanotubes. Steered molecular dynamics studies;* Author: Łukasz Pepłowski.
- Toruń Science and Art Festival – **invited lecture, plenary lecture;** Toruń, Poland (2019-04-24 - 2019-04-28) *Man has not one nam; Man from the point of view of a physicist.* Author: Łukasz Pepłowski.

2018:

- BioInformatics in Torun 2018 - BIT18; Toruń, Poland (2018-06-28 - 2018-06-30) - **poster;** *Docking Studies in Personalized Medicine: Photoactivation of Anti-diabetes Sulfonylurea Drug JB253 and Its Interactions with Epac2 and SUR1 Proteins;* Authors: Łukasz Pepłowski Jakub Rydzewski, Katarzyna Walczeswka-Szewc, Wiesław Nowak.

2017:

- EJTEMM2017 5th European Joint Theoretical/Experimental Meeting on Membranes; Kraków, Poland (2017-12-06 - 2017-12-08) – **poster;** *Interactions of carbon nanotubes with a model cell membrane – steered molecular dynamics studies;* Authors: Łukasz Pepłowski, Jakub Rydzewski, Wiesław Nowak.
- Revolutions in Structural Biology: Celebrating the 100th Anniversary of Sir John Kendrew; Heidelberg, Germany (2017-11-16 - 2017-11-17) – **poster**; *Photoactivation of Potential Anti-diabetes Sulfonylurea Drug JB253 and Its*

Interactions with Epac2 Protein; Authors: Łukasz Pepłowski, Jakub Rydzewski, Tomoo Miyahara, Hiroshi Nakatsuji, Haruki Nakamura, Wiesław Nowak.

- BioInformatics in Torun – BIT17; Toruń, Poland (2017-06-22 - 2017-06-24) – **poster;** *Haptic Device Facilitates Big Data Analysis in Structural Biology;* Authors: Łukasz Pepłowski, Jakub Rydzewski, Wiesław Nowak.

2016:

- BioInformatics in Torun 2016 - BIT16; Toruń Poland (2016-06-16 - 2016-06-18) – **invited lecture;** *Application of the Molecular Dynamics Simulations in Health Related Issues;* Author: Łukasz Pepłowski.

2015:

- BIT15 Bioinformatics in Torun 2015; Toruń Poland (2015-04-16 - 2015-04-18) – **lecture**; *NAMD@GPU;* Authors: Łukasz Pepłowski, Karina Kubiak-Ossowska.

2014:

- BIT14 Bioinformatics in Torun 2014; Toruń Poland (2014-06-12 - 2014-06-14) – **poster;** *TTR related amyloidosis: new insights from simulations and drug design;* Authors: Łukasz Pepłowski, Rafal Jakubowski, Piotr Skrzyniarz, Wieslaw Nowak.
- From Computational Biophysics to Systems Biology 2014 (CBSB14); Gdańsk, Poland (2014-05-25 - 2014-05-27) – **poster;** *Steered Molecular Dynamics Simulations of Constrained and Unconstrained Double Stranded DNA as Explanation of Atomic Force Microscopy Results at High Force Regime;* Authors: Łukasz Pepłowski, Janusz Strzelecki, Aleksander Balter, Wieslaw Nowak.

2013:

- 9th European Biophysics Congress EBSA2013; Lisbon, Portugal (2013-07-13 - 2013-07-17) – **poster;** *Electric field effects on EGF and bFGF ligand- receptor unbinding investigated by Steered MD method;* Authors: Łukasz Pepłowski, Joanna Estkowska, Wiesław Nowak.
- BIT13 Bioinformatics in Torun 2013; Toruń Poland (2013-06-26 - 2013-06-29) – **poster;** *Molecular dynamics study of the βNeurexin-Neuroligin adhesion interactions;* Authors: Łukasz Pepłowski, Rafał Jakubowski, Wiesław Nowak.

2012:

- Modeling & Design of Molecular Materials; Wrocław, Poland (2012-09-10 - 2012-09-14) – **lecture;** *Molecular dynamics studies of synaptic adhesion neuroligin/neurexin complexes;* Author: Łukasz Pepłowski.

- Computer Simulation and Theory of Macromolecules; Hunfeld, Germany (2012-04-20 - 2012-04-22) – **poster**; *Ligand-receptor unbinding in electric fields. MD and Steered MD studies of EGF and FGF growth factors;* Authors: Łukasz Pepłowski, Joanna Estkowska, Wiesław Nowak.
- II Copernican Symposium of Students of Natural Sciences; Toruń, Poland (2012-03-09 - 2012-03-11) – **invited lecture;** *Computer aided modeling of biomolecules - peeping at and improving Mother Nature;* Author: Łukasz Pepłowski.

2011:

- EBSA11, 8th European Biophysics Congress; Budapest, Hungary (2011-08-23 - 2011-08-27) **– poster;** *Molecular dynamics study of bNeurexin-Neuroligin interactions;* Author: Łukasz Pepłowski, Rafał Jakubowski, Adrian Jasinski, Wiesław Nowak.
- BIT11 Bioinformatics in Torun 2011; Toruń Poland (2011-06-02 - 2011-06-04) – **poster;** *EGF and bFGF Cell Signaling Pathways;* Authors: Łukasz Pepłowski, Joanna Estkowska, Wiesław Nowak.
- BIT11 Bioinformatics in Torun 2011; Toruń Poland (2011-06-02 - 2011-06-04) – **poster;** *Steered Molecular Dynamics studies of human ß-neurexin1;* Authors: Łukasz Pepłowski, Rafał Jakubowski, Wiesław Nowak.

2010:

- BIT10 Bioinformatics in Torun 2010; Toruń Poland (2010-06-10 - 2010-06-12) – **poster;** *The Strongest Proteins. All Atom Steered Molecular Dynamics Study;* Authors: Łukasz Pepłowski, Mateusz Sikora, Wiesław Nowak, Marek Cieplak.

Lectures in scientific institutions:

2019:

- Seminary of the Department of Materials Chemistry, Adsorption and Catalysis, Faculty of Chemistry, NCU, Toruń Poland, (2019-12-31) – **invited lecture;** *Photoactive compounds in classical molecular dynamics on the example of potential anti-diabetic drugs.*
- Seminary of School of Biotechnology, Jiangnan University, Wuxi, China (2019-10-28) - **invited lecture;** *How to Run Molecular Dynamics Simulations for Difficult Proteins.*

2018:

- Seminary of School of Biotechnology, Jiangnan University, Wuxi, China (2018-10-26) - **invited lecture;** *Photopharmacology and Thermostability Studies Using Molecular Modeling Aproach.*

2017:

- Seminary of School of Biotechnology, Jiangnan University, Wuxi, China (2017-09-13) - **invited lecture;** *Biomolecules and Light - Theoretical Biophysics Point of View.*

2016:

- Seminary of School of Biotechnology, Jiangnan University, Wuxi, China - **invited lecture;** *Applications of the Molecular Dynamics Simulations in Biotechnology, Nanotechnology and Health Related Issues*.

3. Information on participation in organizational and scientific committees at national or international conferences, including the applicant's function.

   Since 2006, I have been a member of the organizing committee of the annual international conference BIT BioInformatics in Torun organized by Faculty of Physics, Astronomy and Informatics, NCU and the Polish Bioinformatics Society. The conference is held every year in Torun (a total of 17 conferences).
   I have been the chairman of the organizing committee of the BIT BioInformatics in Torun conference since 2015 (8 times in total). In 2022, I was also a member of the program committee of this conference.
   In 2014, the conference was associated with the convention of SocBiN (Society for Bioinformatics in Northern Europe) members. Every year, the conference is attended by 50 to 120 participants, of which about 30% are foreign participants.

4. Information on participation in the works of research teams realizing projects financed through national and international competitions, including the projects which have been completed and projects in progress, and information on the function performed in the team.

   - NCN MINIATURA 2 Grant (action): Study with computer modeling methods of thermostability of nitrile hydratase, an important biotechnological enzyme (Grant No.: 2018/02/X/NZ2/00220), 2018-2019; **Project completed**. **PI**. The result of the work

carried out in the project was the development, by means of computer modeling, of a new variant of NHase from the bacteria Pseudococardia Thermophila, exhibiting much higher thermostability and, by the way, increased catalytic activity. The resulting papers are [**H4**] and [**P5**].

- NCN OPUS 12 Grant: Structural determinants of optical control of insulin and neuroligin release by photoactive protein ligands. (Grant No.: 2016/23/B/ST4/01770), 2017-2022; Project completed. Contractor (PI: Prof. Wieslaw Nowak, NCU).
- Ministry of Science and Higher Education grant: Nanomechanics of modular adhesion proteins (Grant No.: N N202 262038), 2010-2013; Project completed. Contractor (PI: Prof. Wieslaw Nowak, NCU);
- Ministry of Science and Higher Education Grant: Dynamics of biomolecules in coarse-grained models (Grant no.: N N202 0852 33) 2008-2011; Project completed. Contractor (NCU: Prof. Marek Cieplak, IF PAS);

5. Membership in international or national organizations and scientific societies, including the functions performed by the applicant.

- Membership in the Polish Bioinformatics Society (since 2008).
- Membership in the Biophysical Society (Rockville, Maryland, USA; since 2022)

6. Information on internships completed in scientific or artistic institutions, also abroad, including the place, time and duration of the internship and its character.

- Research internship in the R&D department of the pharmaceutical company Adamed Sp. z o.o. Pieńków
  01.02 – 31.03 2011r.
- Visiting Professor, School of Biotechnology, Jiangnan University, Wuxi, China.
  13.12 – 20.12 2016 r.
- Visiting Professor, School of Biotechnology, Jiangnan University, Wuxi, China.
  11.9 – 25.9 2017 r.
- Visiting Professor, School of Biotechnology, Jiangnan University, Wuxi, China.
  4.10 – 28.10 2018 r.
- Consulting, University of Tsukuba, Center for Computational Sciences oraz Institute of Applied Biochemistry, Tsukuba Japan.
  29.10 – 4.11 2018 r.
- Visiting Professor, School of Biotechnology, Jiangnan University, Wuxi, China.
  18.10 – 31.10 2019 r.

7. Information on scientific or artistic works reviewed, in particular those published in international journals.

   I have reviewed scientific articles for the following journals:

   - Foreign publishers (17 reviews):
     - Structure (Cell Press, IF 5.871)
     - PROTEINS: Structure, Function, and Bioinformatics (Wiley & Sons, IF 4.088)
     - Methods (Elsevier, IF 4.674)
     - Journal of Molecular Graphics and Modelling (Elsevier, IF 2.942)
     - Chemical Papers (Springer, IF 2.146)
     - Journal of Biotechnology (Elsevier, IF 3.595)
     - Process Biochemistry (Elsevier, IF 4.885)
     - Journal of Agricultural and Food Chemistry (ACS, IF 5.895)
     - Catalysts (MDPI, IF 4.501)
     - Molecular Catalysis (Elsevier, IF 5.089)
   - Polish publishers (2 reviews):
     - The Bulletin of the Polish Academy of Sciences: Technical Sciences (Journals of PAS)

8. Information on participation in European or other international programmes.
   - European Regional Development Fund grant ZIFI - Interdisciplinary Physics and Informatics Team (Grant No.: RPKP.05.04.00-04-001/10), 2010-2012; Project completed. Contractor (PI: Prof. Wiesław Nowak, NCU).

9. Information on participation in research teams realizing projects other than those defined in section II.4.
   - PI of a grant funded under the "Excellence Initiative - Research University" program at NCU: "Studies by quantum calculations of factors activating the modified GabR receptor", carried out in 2021-2022. The team includes a doctoral student (I am her associate supervisor) Julia Berdychowska. Budget 25,000 PLN.
   - Co-PI in the "Emerging Research Field". "Nanoscale Biophysics" was established within the "Excellence Initiative - Research University" program at NCU. The competition was aimed to the research teams operating at the Nicolaus Copernicus University in Torun, which in the last five years have demonstrated scientific activity - obtaining funds for scientific research, carrying out research projects and activities to increase the internationalization of research at the University. As the PI of one of the

three scientific groups in the research team, I will manage the work of 4 members and a budget of 330,000 PLN from 2023 to 2026. PI of the entire emerging research field: Dr. Karolina Mikulska-Rumińska, NCU; budget for 2023-2026: PLN 1,000,000. There are 14 people in the emerging research field "Nanoscale Biophysics".

- Member of the Priority Research Team "Medically oriented Molecular Biophysics", established within the framework of the "Excellence Initiative - Research University" program at NCU (2019-2022). The call aimed to identify priority research areas and strategic international partners. The team consisted of 9 people, Leader: Prof. Wiesław Nowak NCU
- Contractor in the research grant "ANTICO - Virtual AFM as a tool for testing ANTI-COvid drugs" funded under the "Excellence Initiative - Research University" program at NCU and conducted from 2020-2022. Leader, Prof. Wiesław Nowak, NCU.

10. Information on membership in the teams assessing applications for financing of research projects, applications for scientific awards, applications in other competitions of scientific or didactic character.
    - As the coordinator of the NAWA-funded PROM program at NCU in 2019-2022, I was the committee leader (8 people in total) evaluating applications from doctoral students for funding for travel and inbound internships and conference trips. I performed the formal evaluation of more than 100 applications and the merit evaluation of about 75 applications
    - Member of the committee evaluating applications of NCU students and doctoral students in 2020 within the framework of the Grants4NCUStudents, NCU "Initiative of Excellence University Research" competition. I evaluated the applications of students and doctoral students for funding of their research within the research university in the discipline of physics. I evaluated 7 applications.
    - Member of the committee evaluating the best master's and engineering theses defended in 2021 of Technical Physics, conducted at the Faculty of Physics, Astronomy and Informatics, NCU.
    - Twice a selection committee member, evaluating doctoral students for the PhD school of Science and Natural Sciences, NCU, funded by NCN (Sonata 15, PI Dr. Karolina Mikulska-Rumińska; Sonata 17, PI Dr. Jakub Rydzewski).

III. INFORMATION ON COOPERATION WITH SOCIAL AND ECONOMIC ENVIRONMENT

1. List of technological works.

   None

2. Information on cooperation with economic sector.

   Cooperation with pharmaceutical company ADAMED Pharma S.A. The cooperation was first initiated in 2010, which resulted in a short research internship in 2011. During the cooperation with the pharmaceutical company, using the techniques of theoretical molecular biophysics, bioinformatics and chemoinformatics, I searched for dual inhibitors of the interaction of MDM2 and MDM4 proteins with P53, showing anticancer activity in sarcomas, leukemias and lymphomas (currently the project is at the stage of preclinical studies). The knowledge gained from the cooperation with the pharmaceutical company resulted in the two computer courses offered to NCU students and doctoral students: “Computer Aided Modeling of Drugs” and “Biological and Medical Databases.” Collaboration on the development of the above drugs ended in 2011. In 2022, I started again (unofficially so far) the cooperation on the study of drugs used in anti-obesity.

3. Obtaining the right of industrial property, including the national or international patents granted.

   None

4. Information on implemented technologies.

   None

5. Information on performed expert analyses or other studies prepared on request of public institutions or entrepreneurs.

   None

6. Information on participation in expert and competition teams.

   None

7. Information on artistic projects realized in non-artistic environment.

   None

IV. SCIENTOMETRIC INFORMATION

1. Information on the Impact Factor (in the fields and disciplines in which this parameter is commonly used as a scientometric index).
    a. Total Impact Factor of publications submitted as a habilitation application: ([**H1**]-[**H8**]): **51,895**
    b. Total Impact Factor of articles published after the doctoral degree: **83,206**
2. Information on the number of citations of the applicant's publications, including a separate list of self-citations.
    a. Citations by GS: **244 (209** without self-citations**)**
    b. Citations by WoS: **181 (152** without self-citations**)**
3. Information on h-index held.
    a. Hirsch index by GS: **10**
    b. Hirsch index by WoS: **9**
4. Information on the number of the points awarded by the Ministry of Science and Higher Education.
    a. Number of points of the Ministry of Science and Higher Education (based on the list published in the announcement of December 21, 2021) of publications submitted as a habilitation application: ([**H1**]-[**H8**]): **940**
    b. Number of points of the Ministry of Science and Higher Education (based on the list published in the announcement of December 21, 2021) of articles published after the doctoral degree: **1 670**

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(Applicant's signature)