



MS11 Final report (summary)

Name of project: **Fostering high scientific quality in protein research in Eastern Slovakia (CasProt)**

No. 952333

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Summary of the context and overall objectives of the project

What is the problem/issue being addressed?

Liberal economic reforms accompanied by an influx of foreign investment in the early 2000s resulted in strong economic growth that earned Slovakia the nickname the 'Tatra Tiger'. Today, though, the model of low costs combined with cheap, skilled labour seems to have run out of steam, and Slovakia is looking for a new impulse to drive economic growth and help it catch up with its European Union peers. Slovakia is confronted with significant challenges characterized by economic disparities, lower performance in research and innovation (R&I), and a devastating exodus of its invaluable, highly skilled workforce, commonly known as the "brain drain." Notably, Eastern Slovakia experiences an unusually high emigration rate while contributing only 14% to the overall Slovakian GDP. But while experts say innovation is the best way to go, Slovakia's current innovation capacity is low, as judged by international comparisons. The project CasProt with the coordinating organization Pavol Jozef Šafárik University in Košice (UPJŠ) will provide excellent support for scientific development with an intention to mitigate the brain drain from Eastern Slovakia. A general objective of the CasProt project is to significantly strengthen the scientific excellence in protein science at UPJŠ by linking it with two internationally leading institutions from an EU Member State and an Associated Country: Technical University of Munich (Germany) and University of Zürich (Switzerland).

Why is it important for society?

The success of the project will unavoidably lead to a higher reputation and networking channels of coordinating institution UPJŠ. We suppose that UPJŠ will become a leader in the field of protein science in Slovakia after the project implementation, with the potential to attract and incorporate recognized as well young promising scientists/early-stage researchers into UPJŠ and in the long-term to slow down or even reverse the brain-drain from Slovakia.

The combination of the financing from the CasProt project and from the European Structural Funds has already created a synergy effect leading to a powerful enhancement of the capability of UPJŠ to compete successfully with the internationally leading institutions for EU and international research funding.

Successful outcomes of the project's scientific activities will lead to establishment of protein science cutting-edge technologies (single-molecule techniques and directed protein evolution methods) in laboratories at UPJŠ. These will significantly increase UPJŠ potential, particularly the scientific group at Center for Interdisciplinary Biosciences in networking and collaboration leading academic institutions in Europe and (ii) development of proteins and enzymes for biotechnological and pharmacological purposes.

The connection of the project actions with the educational process at UPJŠ will constitute a platform for creating a unique ecosystem of interdisciplinary-oriented education in bioscience at UPJŠ and, in the future, in Slovakia. Early-stage researchers and pre-graduate students will undoubtedly profit from creating such an educational ecosystem. In summary, the CasProt project can significantly contribute to the long-term sustainability of excellent research, technology transfer, and raise research profile of UPJŠ, particularly in the specific and ambitious fields of single-molecule protein folding and stability, directed evolution of proteins and, potentially, drug delivery system development.

What are the overall objectives?

The main idea behind this project is that the most effective way how to increase the quality of the research in a particular field of science is a collaboration on the joint project with the best laboratories in this field. To this end, we decided to establish the partnership with top universities in Germany and Switzerland - Technische Universität München (TUM) and the Universität Zürich (UZH) – in the field of protein science. Moreover, we believe that this partnership within the CasProt project can unfold the innovation potential of UPJŠ with an impact on the Eastern Slovakia region and Slovakia as a whole.

Concrete main objectives are:

- Reinforcement of scientific capacity and raise the research profile of the coordinating institution UPJŠ in the field of protein science.
- Identification and integration of the experienced and young scientists/early-stage researchers at UPJŠ.
- Enhance and stimulate the technological capacity of the coordinating institution UPJŠ.
- Strengthening the research management and administration skills at UPJŠ.

Progress beyond the state of the art, results and impacts of the project

New technologies and methods have been established in the laboratories of UPJŠ, such as the protein evolution method – ribosome display. As a result of the collaboration with the UZH group, we are in the process of establishing the yeast display method, which represents another powerful method of the directed protein evolution. Practical skills obtained during the stay at UZH initiated an establishment of new method of the expression and purification of eukaryotic proteins based on the baculovirus expression system (BEV). This method expands our capability to express different eukaryotic proteins, difficult to express in bacterial systems, in CIB laboratories.

Study stays at TUM resulted in deepening of knowledge of utilization of the state-of-the-art single molecule technique – optical tweezer. Obtained skills regarding preparation of nanodiscs provide a unique opportunity to study biophysical properties, such as dynamics and stability of integral membrane proteins, such as GPCR, in their natural environment.

Support of our research by project CasProt and projects from the EU structural funds OPENMED (Budget for UPJŠ: 4.9 million EUR) and BioPickmol (Budget for UPJŠ: 2.1 million EUR) as well as by projects supported by Slovak grant agencies APVV and VEGA had a synergic effect and resulted in opening of new laboratories, which have been equipped with the state-of-the-art instruments worth over 1 million EUR.

Thanks to the CasProt support, we have established new collaborations with the top laboratories in the field of rational design - the group of Prof. Damborsky at Loschmidt laboratories at Masaryk University in Brno in Czech Republic and Prof. Fraaije group at University of Groningen in Netherlands. Very recently, we have submitted joint project on the topic related to the utilization of ribosome display in improving properties of thrombolytics based on staphylokinases with vast potential in clinical application, haloalkane dehalogenases with potential in bioremediation and genetically encoded photosensitizers with potential in photodynamic therapy.

In conclusion, the CasProt project significantly contributed to the long-term sustainability of excellent research, technology transfer, and raise research profile of UPJŠ. The collaboration with the partner groups at UZH and TUM resulted in new collaborations with groups at Comenius University and Neuroimmunology Institute of Slovak Academy of Sciences in Bratislava (Slovakia), Loschmidt laboratories at Masaryk University in Brno (Czech Republic), University of Groningen (Netherlands) and University of Bayreuth (Germany). CasProt project thus contributes to the enhancement of the R&I performance of Slovakia and significantly subsidizes the creation of highly competitive research institutions in Eastern Slovakia and by this means strengthens the economic potential and social cohesion of this region, fully in agreement with the main priorities of the Slovak government.

The summarizing report on all actions, activities and achievements of the CasProt project

The [project website](#) is available to the broadest public and it reflects actual project actions and activities such as scientific workshops, symposia, summers schools, conferences, publications as well as all the news and achievements associated with the project. It also contains the information about the project, project coordinator and coordinating partners, research activities, laboratories, media and news. The electronic version of the [promotional materials as well as templates](#) of the project documents can be downloaded from the webpage. The website was launched on December 11, 2020, and since then has been regularly updated and reorganised as needed.

Collaboration between our groups at UPJŠ and groups at partner universities, the group of Prof. Plückthun at UZH and the group of Prof. Rief at TUM, has been significantly strengthened, especially in the second period when we were able to fruitfully accomplish all the aims established within the CasProt project. The most important fact was that after the long pandemic periods of COVID-19 (which significantly affected the project implementation in the first period), we were able to realize all the planned as well as postponed events and actions.

Research activities and know-how transfer

The achievements of know-how transfer and research activities realized within the CasProt project are summarised [elsewhere](#). The report addresses the know-how increase in four specific topics of the directed evolution of GPCRs: (i) Selection and ad hoc design of suitable GPCR candidates; (ii) Expression and purification of GPCRs (e.g., -opioid receptor, NTR); (iii) Rational identification of hot spots in selected GPCRs for evolution; and (iv) Application of protein evolution methods, e.g., yeast display and ribosome display, to enhance the solubility of proteins. Besides, the report further addresses three specific topics related to the development of single-molecule force assays: (i) technical assistance and know-how support in the development of specific biological assays for membrane proteins, preparing materials and modifications for single-molecule experiments; (ii) hands-on design and case study of instrument development for single-molecule force and fluorescence applications; and (iii) advanced instrument and software development for force and fluorescence experiments with selected GPCRs.

Incoming and outgoing visits

The aim of Task 1.6. Outgoing visits, planned and realized within WP1: research activities and know-how transfer, was to reinforce the expertise of the UPJS team in all scientific areas defined and solved in the CasProt project. At the same time, it should have supported the contribution of knowledge and expertise brought by experts within Task 1.5. Incoming expert visits. However, the pandemic situation has remarkably changed the plans as well as the initiation of the CasProt project implementation. Thus, both incoming expert visits as well as outgoing visits from UPJS to the partners'

universities had to be postponed. At the moment, when the situation allowed it (approximately in the middle of the year 2021), we started to realize the planned outgoing visits, and the majority of the outgoing visits took place in the second half of the project. Thus, until the end of the CasProt project, 8 senior scientists and 5 PhD students visited partnering organizations, the University of Zürich (UZH) and the Technical University of Munich (TUM), for short- or long-term stays, some of them repeatedly. They spend there in total approximately 18 PMs.

Regarding the content of these visits, UZH and experts belonging to and working at this university (Prof. A. Plückthun and his group) offered the researchers from UPJŠ the required know-how through lectures, consultations, and discussions, as well as provided conditions for full-fledged practical training focused on gaining advanced knowledge in directed molecular evolution techniques, represented by methods of display technologies such as ribosome, phage, and yeast displays, and in the development of proteins for biotechnological and pharmaceutical applications. Similarly, at TUM (Prof. M. Rief and his group), both senior scientists and young researchers could gain and reinforce their knowledge in all aspects of single-molecule measurements of proteins using high-resolution optical tweezers. Using this method is especially important since it allows the unfolding and refolding of the molecule of interest several times by successively stretching and relaxing the protein.

The outgoing visits also served to report the progress of the CasProt project implementation, manage the incoming events (symposia, workshops, incoming expert visits, etc.), prepare common publications, and discuss plans for the next projects. The theoretical and practical knowledge gained during these stays in specific areas of protein science also helped to set up the techniques listed above in the shared laboratories of the CIB and the Department of Biophysics in Košice. Actually, one of the visible impacts and outputs related to the realization of the reviewed task was the opening of the renovated laboratories of CIB TIP-UPJŠ and the Department of Biophysics UPJŠ in Košice, in which these relevant techniques were installed and are/are going to be used.

Task 1.5. Incoming expert visits was greatly affected in the first half of the project by the pandemic, which hampered travel and the implementation of activities. Therefore, visits by experts from the two partner universities were carried out in the second half of the project. Four incoming experts from UZH and two from TUM took part in this task. Prof. Andreas Plückthun and the experts from his team at UZH trained us in the use of directed molecular evolution techniques, and Prof. Matthias Rief and his team from TUM trained us in single-molecule proteins, folding and refolding.

In particular, they imparted their knowledge to us in fundamental contributions that enabled the emergence of antibody engineering, in particular through the use of *E. coli* as an engineering platform, studies on synthetic antibodies that led to the first fully synthetic antibody library, the development of ribosome display (a true in vitro protein evolution technology), and the development of Designed

Ankyrin Repeat Protein (DARPin) technology. Our experts and students were trained in the development and preparation of engineered proteins and applications that were previously very difficult or even impossible. The focus was on biological areas such as tumour targeting, gene therapy with engineered viruses, G protein-coupled receptors, and a modular detection technology for linear epitopes. Based on the knowledge acquired by the UZH experts, two laboratories were set up during the implementation of the project: (i) the yeast display laboratory for the production of proteins in yeast, and (ii) the insect cell laboratory for the production of eukaryotic proteins in insect cells.

The experts from TUM have provided us with their expertise in the field of single-molecule protein folding, the use of high-resolution optical tweezers to study protein folding processes and molecular machines. Using single-molecule measurements on proteins, it is possible to directly observe and manipulate the protein motions and study how they are affected by interactions with other molecules. From such experiments, it is also possible to determine the rates of folding, unfolding, association and dissociation, as well as the stability of proteins under different conditions. Due to the conservation of momentum, it is possible to capture microscopic particles using sharply focused laser beams. The object trapped in the laser focus obeys Hooke's law, according to which the restoring force felt by the object is linear to its displacement from the trap centre. The focused laser beam can thus be considered a spring for microscopic objects. Based on the knowledge acquired by the experts from TUM, the optical tweezers laboratory was set up and upgraded.

Workshops and symposia

Workshops

The [Single Molecule Folding Workshop](#) took place from the 13th to the 17th of March 2023 and was organized as a joint event by UPJŠ and TUM in Antholz, Italy. The workshop aimed to bring together experts in the field to discuss various topics and aspects related to single-molecule protein folding. The 5-day workshop was dedicated to discussing the latest advancements in single-molecule protein folding research and applied areas. Participants included partners from the organizing institutions as well as invited specialists from external organizations. The discussions covered recent prospects and perspectives in single-molecule protein biophysics, with a particular focus on advancements in related instrumentation and scientific areas. Key topics explored during the workshop included interactions within biological systems, protein-protein mechanics, and the construction and design of optical tweezers and microfluidic devices. These discussions aimed to deepen participants' understanding of single-molecule protein folding, as well as to address the fundamental aspects of protein-protein interactions and instrument design. UPJŠ were represented at the symposium by dr. Žoldák and 2 PhD students.

The [Workshop on Directed evolution of proteins](#) took part in Košice in the CIB at Jesenna 5, Košice from May 2nd to May 5th 2023. The intention of the workshop was to get together experts on protein evolution and on protein design and particularly on protein expression, the topic very closely related to evolution and design new, very often unstable and hard-to-express and purify proteins. The main speaker with the introductory plenary lecture was Prof. Dr. Andreas Plückthun from the partner's laboratory of UZH. We took the opportunity to utilize the Workshop for networking with colleagues from the Czech Republic (3 speakers - BIOCEV institute in Prague, Palacky University in Olomouc, and Masaryk University in Brno) and Comenius University in Bratislava (1 speaker) and Neuroimmunology Institute of Slovak Academy of Sciences in Bratislava (1 speaker). As the workshop took place at the same time as the Summer school on Directed evolution of proteins, whereas numerous students used the opportunity to attend the lectures. This "joint" meeting has been accepted with great satisfaction from both speakers and audience point of views. Lectures on the Workshop consisted of 9 lectures of principal investigators and three lectures of senior researchers.

Symposia

A [symposium on protein stability and folding](#) was organized in Hohenkammer between 22-24.5.2022 and in Garching b. Munchen between 25-26.5. 2022. In Hohenkammer, 21 talks were presented by 12 invited principal investigators (PIs), six postdocs and 3 PhD students. Principal investigators are part of the collaborative research center SFB863, devoted to forces in biomolecular systems. Each PI provides unique expertise and viewpoints on different aspects of biomolecules and their super assemblies. At the same time, experimental and theoretical approaches are combined to achieve a high level of synergy, as indicated by a strong publication track record. The number of early-stage researchers was 12, and the CasProt project was presented by project coordinator Assoc. prof. Erik Sedláč, co-investigator prof. Matthias Rief, as well as senior researchers Dr. Abhigyan Sengupta and Assoc. prof. Gabriel Žoldák and three PhD Students from UPJŠ in Košice. After the first part of the symposium, the event moved to Garching, Center for Protein Assembly and continued with the introduction of laboratories in Košice. During the symposium, a new collaboration was established with prof. Martin Zacharias regarding the availability of their computing time at their high-performance cluster.

The [Symposium on Directed evolution of proteins](#) took part in Tatranská Lomnica in Gran Hotel Praha from April 28th to May 1st 2023. The intention of the symposium was to get together experts on protein evolution and on protein design in general and protein characterization by state-of-the-art techniques such as a single molecule spectroscopy. The experts were invited from the partner's laboratories of UZH (4 persons) and TUM (1 person) within the project CasProt as well as 2 principal

investigators from laboratories collaborating on different projects with the laboratories at Center for Interdisciplinary Biosciences (CIB), i.e. Loschmidt laboratories at Masaryk University in Brno, Czech Republic and Neuroimmunology Institute of Slovak Academy of Sciences in Bratislava, Slovakia. Of 25 symposium participants, 6 were the principal investigators of the group at CIB and 10 early-stage researchers on different stage (PhD student, postdocs, young researcher) of their research careers. Lectures on the Symposium consisted from 9 lectures of principal investigators and 4 lectures of early stage researchers. The program and the location of the Symposium offered numerous possibilities for discussions of young researchers with the principal investigators as well as for establishing new connections and networking among principal investigators.

Summer schools

The [Summer school on protein stability and folding](#) was organized by UPJŠ and involved three Departments of two Institutes: The Department of Biophysics, the Institute of Physics, the Department of Biochemistry, Institute of Chemistry of the Faculty of sciences. The executive management and coordination of Summer school were in the hands of the Center for interdisciplinary biosciences (CIB), Technology and Innovation Park TIP-UPJS. An overall 11-day summer school on protein stability and folding consisted of several parts: the first part consisted of lectures by Slovakian and foreign researchers, which were realized during 27-28.06.2022. The second part was focused on experimental work between 29.06-4.7.2022. During this phase, the participants were split into two groups, from which one group conducted experiments at CIB, and the second group conducted experiments at the Department of Biochemistry. After two days, the groups switched locations for another two days. The third part of Summer School was devoted to hands-on theoretical training on 06.07.2022. On the last day of the summer school, all experimental results and selected publications were presented by Summer school participants. All participants had the chance to choose a research paper from protein stability and folding and present the research paper to the audience. Positive and constructive feedback was given by Assoc. Prof. Gabriel Žoldák and Assoc. prof. Rastislav Varhač. The total number of participants was 11; ten participants were able to finalize presentations and participate in experiments. Overall, six early-stage researchers were from UPJŠ and three students from Comenius University in Bratislava and one from scientific institutions – Slovak Academy of Sciences, Košice. Lectures and talks were held in English. We would like to emphasize the presence of two invited speakers Dr. Abhigyan Sengupta from TU Munich, Germany, who attended the meeting in person. The second speaker, Dr.med. Magdalena Harakalova from University Utrecht, Netherland, joined the Summer School online using the WEBEX application.

We would like to emphasize that an utterly new event was established thanks to the CasProt project. We plan to organize Summer School on Protein stability and folding regularly – the next Summer school is scheduled for 2024.

The [Summer School on Directed Evolution of Proteins](#) was held for the first time at the CIB, TIP-UPJŠ in Košice from May 2nd to May 12th, 2023. The school provided participants with a comprehensive understanding of protein evolution and expression and purification. The program integrated practical laboratory sessions with lectures delivered by experts in the field. The primary focus of the Summer School was to introduce participants to the efficient protein evolution method known as a ribosome display. The students had the opportunity to learn the fundamentals of this technique by actively engaging in all the necessary experimental steps. The Summer School also included lectures delivered by researchers from Pavol Jozef Šafárik University, Universität Zürich, and the Slovak Academy of Sciences. These lectures covered a wide range of topics related to protein evolution, including protein engineering strategies and the principles of directed evolution. Eight students actively participated in the Summer School. The program spanned two weeks, with each week focusing on different aspects of protein evolution and engineering. During the first week, students were introduced to various methods including error-prone PCR, restriction digestion, ligation, or cell transformation. Notable lectures were given by Andreas Plückthun, world-recognized scientist and professor in the field of protein engineering and directed evolution, who along with his research team, has made significant contributions to the development and optimization of the ribosome display technique. The first week concluded with laboratory work sessions. All laboratory sessions were led by researchers working at CIB TIP UPJŠ, namely Ľuboš Ambro and Mária Tomková. The second week of the Summer School focused on specific laboratory work related to plasmid isolation, preparation for ribosome display, and the actual practice of ribosome display. Participants were engaged in intensive laboratory sessions, allowing them to apply their theoretical knowledge and practical skills in the context of directed protein evolution. The Summer School ended up with presentations and closing remarks on Friday, May 12th. Participants had the opportunity to showcase their work and share their findings from the practical sessions conducted throughout the program. The presentations provided a platform for students to demonstrate their understanding of the ribosome display technique and its applications in directed protein evolution.

Overall, the Summer School on Directed Evolution of Proteins offered a comprehensive learning experience to the participants, combining lectures by experts with hands-on laboratory sessions. The integration of theory and practice provided students with the necessary skills and knowledge to pursue further research in the field of protein engineering.

The [Summer School on biophotonics](#) was organized by doctoral school of UPJŠ and was held at the Department of Biophysics (Institute of Physics, Faculty of Science, UPJS) and CIB, TIP-UPJŠ from May 22nd to May 31st, 2023. The executive management and coordination of the school were in the hands of the Department of Biophysics. An overall 10-day course provided participants with a comprehensive understanding of fundamentals and advanced methods of biophotonics. The program integrated practical laboratory trainings with lectures/theoretical knowledge delivered by Slovak and foreign researchers and invited experts in the field. The primary focus of the Summer school was to introduce participant to the recent advances in biophotonic research and corresponding efficient techniques which open new possibilities of non-contact, high-speed, multi-dimensional measurements and precision imaging methods. The students had the opportunity to learn fundamentals of these techniques within a set of lectures dedicated to optical spectroscopy, imaging and time-resolved techniques. The students were also provided with dedicated lectures on Safety and Good Laboratory Practice, as well as Ethics Issues in biophotonics. The lectures were coherently supplemented with practical demonstrations of the respective experimental techniques. Moreover, the interdisciplinary teams (each of 4-5 students) were formed to develop their own experimental projects, related to the focus of the School. At the end of the summer school, the experimental projects were publicly defended by the students, and subsequently, the students were awarded with the Certificates of attendance. The School of Biophotonics was attended by 14 PhD students from different Slovak Universities, the lectures and practical training were impacted by 10 (inter)nationally recognized experts in the field of biophotonics (invited foreign speakers include prof. H. van den Bergh, dr. G. Wagnieres and dr. S. Sanchez-Cortes; Slovak researchers and experts include prof. P. Miškovský, prof. A. Marček Chorvátová, dr. D. Chorvát, dr. G. Žoldák). All laboratory sessions were led by researchers working at the Department of biophysics and Center for interdisciplinary biosciences, namely Gregor Bánó, Zuzana Jurašková and Veronika Huntošová.

In summary, the Summer school of biophotonics was highly acclaimed by all of the participants, as well as lecturers. The student's teams presented high-quality research projects that we believe can lead in future to new collaborations, proposals and research projects.

On-job trainings

At the end of the 2022 year, the on-job trainings of the UPJS administrative staff were realized to reinforce their management and administrative skills. In total, 7 persons (2 x project managers, 2 x financial managers, 2 x advisors for Horizon Europe projects), mainly from the "Project administrative office" (CCVaPP), but also members from TIP-UPJŠ, visited the partners' universities TUM (October 24th – 28th, 2022) and UZH (November 28th – December 2nd, 2022). They were trained in/obtained advanced knowledge/information regarding the issues and field of the scientific management and

administration: finding the proper calls, partners search skills improvement, improving deliverables preparation skills, improvement project and financial management skills, understanding of the scientific agencies' role in ERA.

Communication and dissemination

The [project website](#) remained the main/leading information, communication and dissemination resource concerning the project (implementation) and relevant activities. Besides, social media ([Facebook](#), [LinkedIn](#), [Twitter](#)) were also recognized as an effective channel to spread/distribute the project knowledge and results. We were/are managing to keep them continuously updated and active.

Preparation of the particular CasProt project YouTube channel has not been shown to be effective, therefore we used to share the [promotional video](#) through the [UPJŠ YouTube channel](#). Further, different conference presentations, workshops, webinars, seminars, open door events, press and public releases, etc. were also an important tool for the project promotion. The [created logo, ppt presentations and two informative roll-up banners](#) of the CasProt project were regularly used at different events and presentations. For each of the events organized within the CasProt project we prepared different posters, programs and/or leaflets. Moreover, customized promotional products (bags, pens, notebooks, and USB drives) were designed, produced and provided mainly to the CasProt events' (symposia, workshops, summer schools) participants.

During the project implementation we prepared and published: [5 press releases, 5 promotional articles, 1 promo video, 2 interviews within the scientific tv magazine produced by national television, and 1 podcast](#). Publication of some of these activities/information was multiplied by its distribution through different media sources (newspapers, journals, TVs, etc.). After the CasProt project realization, we have not finished to promote the project, and actually, we have prepared and submitted for publication/are preparing other promotional articles in national media sources as well as in the EU platform (Horizon/Futuris Magazine).

Conferences

One of the task of the WP4 was to enhance the scientific recognition of the CIB TIP-UPJS group by the attendance of the most prestigious conferences in biophysics, biochemistry, and protein science.

In the first half of the project, the CasProt project financially supported 11 conference participations (5 senior researchers, 6 PhD students): 7 x **13th European Biophysics Congress**, Vienna, Austria, July 24-28, 2021; 3 x **XXVI. Annual Congress of Czech and Slovak Societies for Biochemistry and Molecular Biology with cooperation of Austrian and German Biochemical Section**, České Budějovice, Czech Republic, August 29- September 1, 2021; 1 x **Interfaces 2021**, Santa Margherita di PULA, Sardinia, Italy, September 21-25, 2021.

In the second half of the project, we were able to financially support 17 conference attendance (11 senior researchers, 6 PhD students): 2x **Instruct Biennial Structural Biology Conference**, Utrecht, The Netherlands, May 18-20, 2022; 2 x **21st European Bioenergetics Conference**, Aix-en-Provence, France, August 20-25, 2022; 10 x **Regional Biophysics Conference**, Pécs, Hungary, August 22-26, 2022; 2 x **16th Multinational Congress on Microscopy**, Brno, Czech Republic, September 4-9, 2022; 1x **Odpadové fórum 2022**, Hustopeče, Czech Republic, September 20-22, 2023.

There is a brief summary of the conferences participation supported by the CasProt project:

	Task 4.5. FIRST HALF	Task 4.6. SECOND HALF	Σ	
No. of conferences:	3	5	8	
No. of oral presentations	2	5	7	
No. of poster presentations	9	12	21	
No. of participating senior scientists	5	11	16	28
No. of participating PhD students	6	6	12	

Besides the presentation and dissemination of the results obtained during the CasProt projects, the participants (the UPJŠ team) also have the opportunity to be in touch with up-to-date research in the fields of protein science, biochemistry, and biophysics, as well as related scientific fields, and to meet new people and establish new contacts and collaborations with other experts and leading groups in the mentioned scientific fields. Moreover, they also had a unique opportunity to visit and learn about the history, actual organization, and functioning of the corresponding foreign universities, institutions, and laboratories where the conferences took place.

Finally, it is important to note that support of our research by CasProt project, projects of European Structural Funds (OPENMED and BioPickmol) and projects supported by Slovak grant agencies APVV and VEGA had a synergic effect on the obtained results presented in 14 lectures and 22 posters at national and foreign conferences. Besides, in the 2nd period of the CasProt project implementation, the symposia and workshops were also organized. Within these actions, members of our teams have presented 17 lectures and 3 posters for the events participants. For more information about the programmes of these events you can see the corresponding deliverables - Symposia and workshops (Deliverables 2.1, 2.2, 2.3, 2.4). All these presentations are listed below:

Lectures (32):

(1) Petrenčáková M., Filandr F., Hovan A., Man P., Kožár T., Novák P., Jancura D., Bánó G., Sedlák E.: Flavin cofactor in AsLOV2 domain as efficient singlet oxygen producer. XXVI. Annual Congress of

Czech and Slovak Societies for Biochemistry and Molecular Biology with cooperation of Austrian and German Biochemical Section, 29. august-1. september, 2021, České Budějovice, Czech Republic.

(2) Žoldák G., Džupponová, V.: Salt-dependent antibody adsorption on water/solid interfaces. International Conference on Interfaces, From new materials to life science – Structure, Interactions, Dynamics and Activity, 21. – 25. september 2021, Santa Margherita di PULA (CA), Sardinia, Italy, O15.

(3) Talafová V., Humeník M., Žoldák G., Scheibel T., Sedlák E.: Samozostavovanie rekombinantného pavúčieho proteínu do fibríl vplyvom pH a kozmotropných iónov. Spomienkový seminár pri príležitosti nedožitých 100. narodenín prof. MVDr. Michala Bartíka, DrSc., September 29, 2021, Univerzita veterinárskeho lekárstva a farmácie v Košiciach, Košice, Slovakia.

(4) Džupponová V., Žoldák G.: Úloha disulfidových väzieb amyloidotického ľahkého reťazca IgG pri tvorbe extracelulárnych agregátov. Spomienkový seminár pri príležitosti nedožitých 100. narodenín prof. MVDr. Michala Bartíka, DrSc., September 29, 2021, Univerzita veterinárskeho lekárstva a farmácie v Košiciach, Košice, Slovakia.

(5) Žoldák G.: Development of single-molecule protein biophysics. Instruct-ERIC webinar series: structure meets function – Webinar #13 Instruct Slovakia, October 12, 2021, <https://instruct-eric.eu/content/instructeric-webinar-series-structure-meets-function>.

(6) Lecture entitled "From Snina to the world and back to Košice." As part of the Meet Your Scientist event. The event took place at the invitation of the director of the Grammar School of St. Cyril and Methodius in Snina (Švermova 10, 06901 Snina, mobil: 0911 798 700), September 24th, 2021. The event was attended by 23 students of the Grammar School, Slovakia.

(7) Sedlák E. (invited lecture) Evolution of proteins in a test tube. Evolutionary techniques and their use. Within the cycle Selected Chapters from Molecular Biology (doc. Stanislav Stuchlík, PF UK in Bratislava, Department of Molecular Biology). Košice, November 26th, 2021 (online). The event was attended by 20 students of the Comenius University in Bratislava, Slovakia.

(8) Sedlák E.: Singlet oxygen production by LOV domain. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; L35.

(9) Kubackova J., Slabý C., Horvath D., Hovan A., Iványi G. T., Vizsnyiczai G., Kelemen L., Strejčková A., Tomori Z., Bánó G.: Viscosity measurements using flexible microstructures. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; L20.

(10) Huntošová V.: Time-resolved detection of oxidative stress level in cancer cells. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; L52.

(11) Žoldák G.: Single-molecule mechanics of proteins. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; L14.

(12) Žoldák G.: Mechanics and evolution of Hsp70. Book of Contributions, 10th Slovak Biophysical Symposium, May 3 – 5, 2022, Smolenice, Slovakia, pp. 44, http://confolab.sav.sk/ovsb/zbornik-abstraktov_skbs2022/.

(13) Džupponová V., Žoldák G.: Aggregation mechanism and morphologies of myelomatic human light chain. Book of Contributions, 10th Slovak Biophysical Symposium, May 3 – 5, 2022, Smolenice, Slovakia, pp. 68-69, http://confolab.sav.sk/ovsb/zbornik-abstraktov_skbs2022/ (lecture).

(14) Felčíková, K.; Sedlák, E.: Alternative design of flavoprotein-based photosensitizers. XXVIIth Biochemistry Congress of Slovak and Czech Societies for Biochemistry and Molecular Biology with cooperation of Hungarian and Ukrainian Biochemical Societies. September 10th-13th, 2023, High Tatras, Slovakia, Abstract of lectures.

(15) Sedlák E.: Research topics in Center for Interdisciplinary Biosciences in Košice. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/Garching b. München, Germany.

(16) Žoldák G.: Protein folding and stability – human myeloma light chain proteins. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/Garching b. München, Germany.

(17) Gala M.: In silico protein evolution. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/Garching b. München, Germany.

(18) Dzurillová V.: Utilization of ribosome display in directed evolution of haloalkane dehalogenase DhaA. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/Garching b. München, Germany.

(19) Felčíková K.: LOV2 domain as photosensitizer in photodynamic therapy. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/Garching b. München, Germany.

(20) Nemergut, M.: Identifying a new aggregation hotspot in Alzheimer's disease: Opportunities for drug discovery. Workshop on directed evolution of proteins. 02.05.-05.05.2023, Košice, Slovakia.

(21) Ambro, Ľ.: Production and purification of recombinant proteins: pitfalls and problem solving. Workshop on directed evolution of proteins. 02.05.-05.05.2023, Košice, Slovakia.

(22) Hovanová, V.: From macroscopic measurements to microscopic events. Workshop on directed evolution of proteins. 02.05.-05.05.2023, Košice, Slovakia.

(23) Žoldák, G.: The application of nanomechanical studies for protein engineering. Workshop on directed evolution of proteins. 02.05.-05.05.2023, Košice, Slovakia.

(24) Huntošová, V.: Fluorescence and imaging of proteins in cells. Workshop on directed evolution of proteins. 02.05.-05.05.2023, Košice, Slovakia.

(25) Sedlák, E.: Genetically encoded photosensitizers. Workshop on directed evolution of proteins. 02.05.-05.05.2023, Košice, Slovakia.

(26) Žoldák, G.: Structural mechanics of E. coli Hsp70. Symposium on directed evolution of proteins. 28.04.-01.05.2023, Tatranská Lomnica, Slovakia.

(27) Tomková, M.: Directed evolution of staphylokinase by ribosome display. Symposium on directed evolution of proteins. 28.04.-01.05.2023, Tatranská Lomnica, Slovakia.

(28) Sedlák, E.: Design of genetically encoded photosensitizers. Symposium on directed evolution of proteins. 28.04.-01.05.2023, Tatranská Lomnica, Slovakia.

(29) Dzurillová, V.: Directed evolution of enzymes – the case of HLDs. Symposium on directed evolution of proteins. 28.04.-01.05.2023, Tatranská Lomnica, Slovakia.

(30) Džupponová, V.: Aggregation mechanism of myelomatic human light chain. Symposium on directed evolution of proteins. 28.04.-01.05.2023, Tatranská Lomnica, Slovakia.

(31) Gala, M.: Computational evolutionary analysis of insertion and deletion events in bacterial Hsp70. Symposium on directed evolution of proteins. 28.04.-01.05.2023, Tatranská Lomnica, Slovakia.

(32) Hovanová, V.: Self-assembly of recombinant spider silk protein. Symposium on directed evolution of proteins. 28.04.-01.05.2023, Tatranská Lomnica, Slovakia.

Posters (25):

(1) Tomková M., Berta M., Dzurillová V., Sedlák E.: Preparation of a ribosome extract for an in vitro protein evolution through ribosome display. XXVI. Annual Congress of Czech and Slovak Societies for Biochemistry and Molecular Biology with cooperation of Austrian and German Biochemical Section, August 29 - September 1, 2021, České Budějovice, Czech Republic, P099.

(2) Talafová V., Humeník M., Žoldák G., Scheibel T., Sedlák E.: Self-assembly kinetic model of recombinant spider silk protein EADF4(C16). XXVI. Annual Congress of Czech and Slovak Societies for Biochemistry and Molecular Biology with cooperation of Austrian and German Biochemical Section, August 29 - September 1, 2021, České Budějovice, Czech Republic, P110.

(3) Mikulova L., Pechova I., Stupak M., Fabian M., Jancura D.: Thermodynamics of the formation and decay of the P-type ferryl form of cytochrome c oxidase in the reaction of the oxidized oxidase with H₂O₂. Eur. Biophys. J. 50, P-120 (2021). <https://doi.org/10.1007/s00249-021-01558-w>, 13th European Biophysics Congress, July 24-28, 2021, Vienna, Austria.

(4) Dušeková E., Tomková M., Berta M., Dzurillová V., Sedláková D., Sedlák E.: Hofmeister effect on catalytic efficiency and conformation of 3C protease. Eur. Biophys. J. 50, P-101 (2021). <https://doi.org/10.1007/s00249-021-01558-w>, 13th European Biophysics Congress, July 24-28, 2021, Vienna, Austria.

(5) Talafová V., Žoldák G., Humeník M., Scheibel T., Sedlák E.: Self-assembly of recombinant spider silk protein eADF4(C16) triggered by pH. *Eur. Biophys. J.* 50, P-381 (2021). <https://doi.org/10.1007/s00249-021-01558-w>, 13th European Biophysics Congress, July 24-28, 2021, Vienna, Austria.

(6) Berta M., Dzurillová V., Tomková M., Sedlák E.: Modification of GPCR solubility by ribosome display. *Eur. Biophys. J.* 50, P-99 (2021). <https://doi.org/10.1007/s00249-021-01558-w>, 13th European Biophysics Congress, July 24-28, 2021, Vienna, Austria.

(7) Dzurillová V., Berta M., Tomková M., Sedlák E.: Development of haloalkane dehalogenases by ribosome display. *Eur. Biophys. J.* 50, P-103 (2021). <https://doi.org/10.1007/s00249-021-01558-w>, 13th European Biophysics Congress, July 24-28, 2021, Vienna, Austria.

(8) Džupponová V., Žoldák G.: Role the conformational and colloidal stability on time-dependence solubility of human myeloma antibody λ -light chain. *Eur. Biophys. J.* 50, P-102 (2021). <https://doi.org/10.1007/s00249-021-01558-w>, 13th European Biophysics Congress, July 24-28, 2021, Vienna, Austria.

(9) Slabý C., Kubacková J., Hovan A., Miškovský P., Tomori Z., Bánó G.: The Brownian motion of flexible microstructures – towards viscosity measurements in microfluidic channels. *Eur. Biophys. J.* 50, P-177 (2021). <https://doi.org/10.1007/s00249-021-01558-w>, 13th European Biophysics Congress, July 24-28, 2021, Vienna, Austria.

(10) Rief M., Žoldák G.: Direct observation of chemo-mechanical coupling in the Hsp70 chaperone DnaK by single-molecule force experiments. *Instruct Biennial Structural Biology Conference, Abstract Booklet*, pg. 56; P- Poster No. 40.

(11) Džupponová V., Žoldák G.: Molecular mechanism of myeloma λ -type IgG light chain aggregation. *Instruct Biennial Structural Biology Conference, Abstract Booklet*, pg. 57; P- Poster No. 41.

(12) Jancura D., Pechová I., Mikulová L., Stupák M., Fabián M.: Isothermal titration calorimetric study of the formation and decay of the p-form of cytochrome c oxidase. *EBEC 2022, Biochimica et Biophysica Acta (BBA) - Bioenergetics 1863 Supplement (2022) 148678; S3.P8.*

(13) Tomková A., Sztachová T., Fabián M., Jancura D.: Thermodynamic characterization of ferryl intermediates of bovine cytochrome c oxidase. *EBEC 2022, Biochimica et Biophysica Acta (BBA) - Bioenergetics 1863 Supplement (2022) 148680; S3.P10.*

(14) Tomková M., Dzurillová V., Sedlák E.: Evolution of staphylokinase's binding affinity by ribosome display technology. *Regional Biophysics Conference*, <https://www.rbc2022.hu/i-programme.php>; P64.

(15) Hovan A., Pevná V., Huntošová V., Miskovsky P., Bánó G.: Time-resolved singlet oxygen phosphorescence detection in live cell suspensions. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; P25.

(16) Espina A, Sanchez-Cortes S., Cañamares M. V., Jurašková Z.: Structural in-depth analysis of iron complexes of plant gall polyphenols by optical spectroscopic techniques and DFT calculations. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; P29.

(17) Dzurillová V., Ambro Ľ., Artimovič P., Fecková K., Sedlák E.: HaloTag technology in directed evolution of haloalkane dehalogenase. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; P16.

(18) Felčíková K., Dzurillová V., Hovan A., Bánó G., Kožár T., Sedlák E.: Designing of LOV2 protein into efficient photosensitizer. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; P18.

(19) Slabý S., Kubacková J., Horvath D., Hovan A., Iványi G. T., Vizsnyiczai G., Kelemen L., Žoldák G., Tomori Z., Bánó G.: The viscoelastic behaviour of photopolymer nanowires – experiments and modeling. Regional Biophysics Conference, <https://www.rbc2022.hu/i-programme.php>; P57.

(20) Pevná V., Tomková S., Huntošová V.: Targeted photobiomodulation of cancer cells at 808 nm may improve efficacy of photodynamic therapy. 16th Multinational Congress on Microscopy, Book of Abstracts; pg. 274-275; LS4-P-2498.

(21) Pevná V., Tomková S., Huntošová V.: Imaging of autophagic and mitochondrial proteins in 3D spheroids of U87 MG cancer cells during photodynamic therapy mediated with hypericin. 16th Multinational Congress on Microscopy, Book of Abstracts; pg. 276-277; LS4-P-2499.

(22) Štulajterová, M., Tomková, M., Sedlák, E.: Basic characterization of staphylokinase variants evolved by ribosome display. XXVIIth Biochemistry Congress of Slovak and Czech Societies for Biochemistry and Molecular Biology with cooperation of Hungarian and Ukrainian Biochemical Societies. September 10th-13th, 2023, High Tatras, Slovakia, Abstract of lectures, p. 201-202.

(23) Gala M., Pristaš P., Žoldák G.: Sequence-structure analysis of bacterial heat shock proteins 70. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/ Garching b. München, Germany.

(24) Dzurillová V., Sedlák E.: Utilisation of ribosome display in development of haloalkane dehalogenase DhaA. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/Garching b. München, Germany (poster).

(25) Felčíková K., Dzurillová V., Hovan A., Bánó G., Kožár T., Sedlák E.: Design and isolation of new mutant LOV2 proteins. Symposium on protein stability and folding. 22.05.-26.05.2022, Schloss Hohenkammer/Garching b. München, Germany (poster).

Publications

Increased collaboration between UPJŠ and partners' universities supported and promoted within the CasProt project implementation led to the preparation and publication of several [publications](#) elaborated by us (our groups at UPJŠ), in collaboration with our partners' universities as well as in collaboration with other groups (e.g. Slovak Academy of Science in Košice and Bratislava):

(1) Džupponová V., Žoldák G.: Aggregation mechanism and branched 3D morphologies of pathological human light chain proteins under reducing conditions. *Colloids and Surfaces B: Biointerfaces* 221 (2023) 112983; IF: 5.999; Q1.

(2) Nemergut M., Sedláková D., Fabriciová G., Belej D., Jancura D., Sedlák D.: Explanation of inconsistencies in the determination of human serum albumin thermal stability. *International Journal of Biological Macromolecules* 232 (2023) 123379; IF: 8.025; Q1.

(3) Pevná V., Máčajová M., Hovan A., Bánó G., Meta M., Bilčík B., Palková J., Huntosova V: Spheroidal Model of SKBR3 and U87MG Cancer Cells for Live Imaging of Caspase-3 during Apoptosis Induced by Singlet Oxygen in Photodynamic Therapy. *Biomedicines* 10(9) (2022) 2141; IF: 4.757; Q2.

(4) Pevna V., Horvath D., Wagnieres G., Huntosova V: Photobiomodulation and photodynamic therapy-induced switching of autophagy and apoptosis in human dermal fibroblasts. *Journal of Photochemistry and Photobiology B: Biology* 234 (2022) 112539; IF: 6.814; Q1.

(5) Rief M., Žoldák G.: Single-molecule mechanical studies of chaperones and their clients. *Biophysics Reviews* 3 (2022) 041301; IF: 4.433; Q1.

(6) Žoldák G.: Protein Nanomechanics (Editorial). *Nanomaterials* 12(19) (2022) 3524; IF: 5.719; Q1.

(7) Dušeková E., Garajová K., Yavaşer, R., Tomková M., Sedláková D., Dzurillová V., Kulik N., Fadaei F., Shaposhnikova A., Minofar B., Sedlák E.: Modulation of global stability, ligand binding and catalytic properties of trypsin by anions. *Biophysical Chemistry* 288 (2022) 106856; IF: 3.628; Q2.

(8) Dušeková E., Berta M., Sedláková D., Řeha D., Dzurillová V., Shaposhnikova A., Fadaei F., Tomková M., Minofar B., Sedlák E.: Specific anion effect on properties of HRV 3C protease. *Biophysical Chemistry* 287 (2022) 106825; IF: 3.628; Q2.

(9) Gala M., Pristaš P.; Žoldák G.: Allosteric Inter-Domain Contacts in Bacterial Hsp70 Are Located in Regions That Avoid Insertion and Deletion Events. *International Journal of Molecular Sciences* 23(5) (2022) 2788; IF: 6.208; Q1.

(10) Pevná V., Wagnieres G., Huntošová V.: Autophagy and Apoptosis Induced in U87 MG Glioblastoma Cells by Hypericin-Mediated Photodynamic Therapy Can Be Photobiomodulated with 808 nm Light. *Biomedicines* 9(11) (2021) 1703; IF: 6.081; Q1.

(11) Nemergut M., Škrabana R., Berta M., Plückthun A., Sedlák E.: Purification of MBP fusion proteins using engineered DARPIn affinity matrix. *International Journal of Biological Macromolecules* 187 (2021) 105-112; IF: 6.853; Q1.

(12) Sengupta A., Rognoni L.E., Merkel U., Žoldák G., Rief M.: SlyD Accelerates trans-to-cis Prolyl Isomerization in a Mechanosignaling Protein under Load. *The Journal of Physical Chemistry B* 25(31) (2021) 8712-8721; IF: 2.991; Q2.

(13) Bauer J., Žoldák G.: Interpretation of Single-Molecule Force Experiments on Proteins Using Normal Mode Analysis. *Nanomaterials* 11(11) (2021) 2795; IF: 5.076; Q1.

The collaboration with the top laboratories in the field has led to a relatively steep increase in the quality of papers published by the members of the CIB-TIP UPJŠ laboratories. In fact, while during 2020 there were published 21 papers with the average IF 3.4, in 2021: 36 papers with IF 3.6, in 2022: 28 with 4.3, and in 2023 (up to the end of September) 18 papers with IF 7.2.

Thanks to the CasProt support:

(1) UPJŠ organized a series of webinars [SCIENCE MEETS BUSINESS](#), which were predominantly visited by people from research institutes as well as private high-tech companies.

(2) UPJŠ also organized Symposium of the CasProt, BioPickmol and OPENMED projects entitled [TRENDS IN INTERDISCIPLINARY BIOSCIENCES](#).

(3) During the symposium (Symposium on protein stability and folding was organized in Hohenkammer/Garching), a new collaboration was established with prof. Martin Zacharias regarding the availability of their computing time at their high-performance cluster.

New joint project (InterBioCass) with TUM was prepared in HORIZON-WIDERA-2023-ACCESS-01 call.

(4) The research topics addressed in the CasProt project have recently been supported by several grants from Slovak national grant agencies. Our group, members of the Center for Interdisciplinary Biosciences, in close collaboration with the Department of Biophysics, submitted three grant applications for projects in total 13 million EUR within the recently opened calls for The Plan of Renovation and Resilience in Slovakia. The project CasProt significantly contributed to our ability and capacity to apply for such projects through the reinforcement of the scientific capacity of the Center for Interdisciplinary Biosciences in the field of protein science, in particular in the topic of directed protein evolution.