EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

PROTEOCURE Newsletter

07

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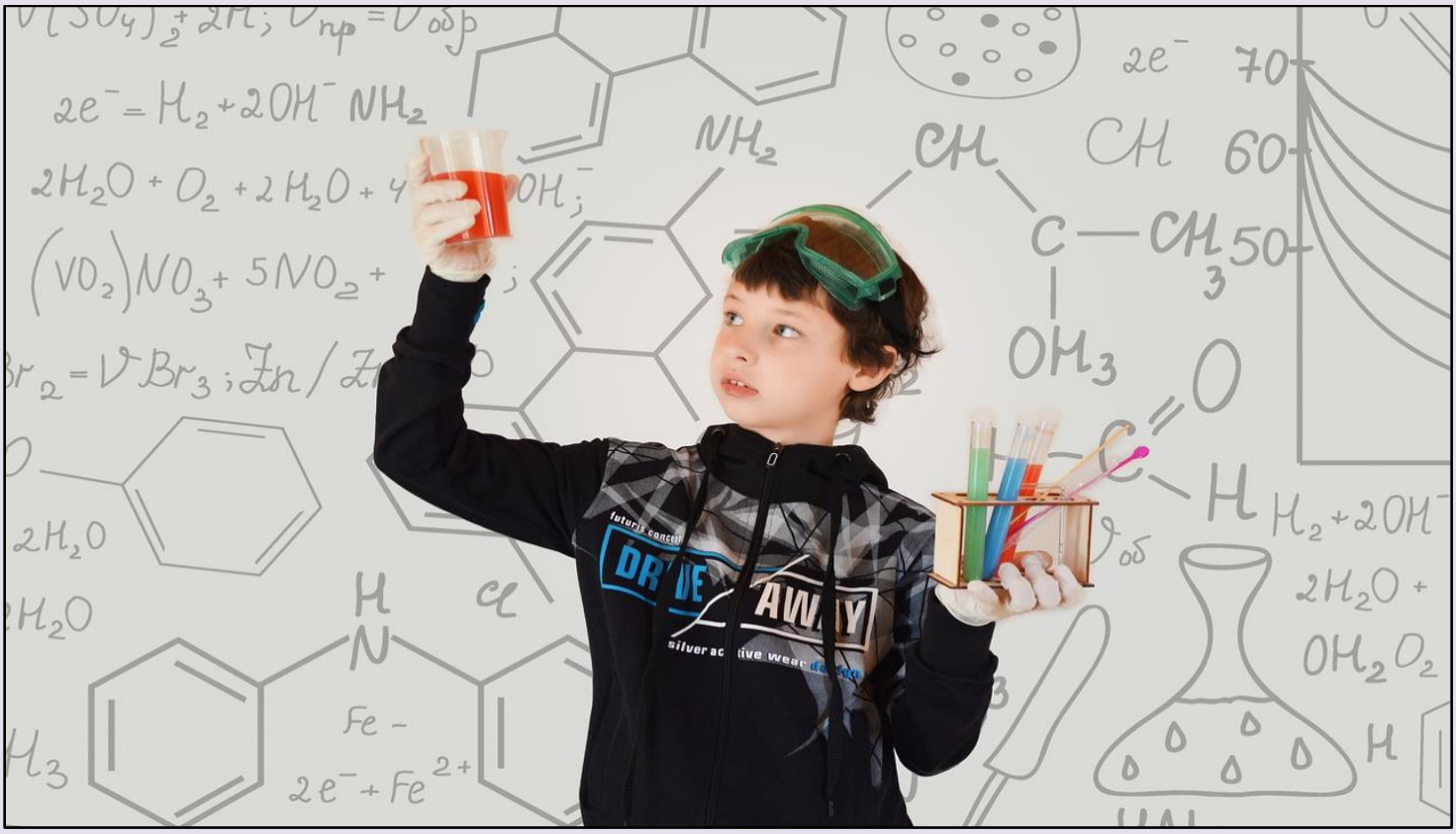
A SOUND PROTEOME FOR A SOUND BODY:
TARGETING PROTEOLYSIS FOR PROTEOME REMODELING



ProteoCURE: impacting early carrier researchers

ACTIVITIES & HIGHLIGHTS

by the ProteoCURE dissemination committee

One of ProteoCure's most important missions is the integration of the multiple aspects of our research area. However, horizontal integration is as important as generational integration. Indeed, we have made the formation of young scientists a priority, as the future belongs to them. It is important not only to transmit training of young scientist but also to share our experience in managing science. This involves aspects such as organising training schools, scientific meetings, communicating with the general public, and fostering collaborations between public and private institutions, among others. ProteoCure dedicates part of its resources to this type of training, committing our efforts to integrate young scientist in our exchange activities. In this special issue, we have contacted three young scientists who have benefited from short-term scientific mission (STSM) grants to learn about the impact this experience had on their projects and careers. They have answered some questions to illustrate their point of view on this training experience. These experiences will be enriched by our upcoming annual meeting, which will take place in early May 2024 in Warsaw. This will be an excellent opportunity for them to meet ProteoCure scientists and discuss their latest results, but also to seek collaborations that will impact their careers in the short and long term. Hoping to see you all there.

Founded by the European Union

COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

COMING SCIENTIFIC EVENTS

ProteCURE ANNUAL MEETING 2024: FINAL ANNOUNCEMENT

The 3rd PROTEOCURE Annual Meeting. Warsaw, Poland. 7-10, May 2024.

You can get all logistic information in our conference website:https://proteocure2024.sciencesconf.org

The final program will appear soon in our conference web site. Don't forget to register to our social events.

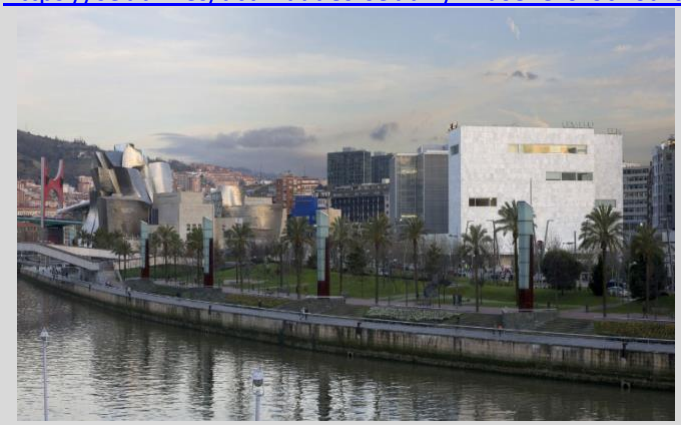
Organizing Committee: Oliver Coux (ProteoCure Chair), Rosa Farras (ProteoCure Vice-Chair), Christine Blattner, Efthimios Skoulakis, Justyna McIntyre (Local Organizer), Ulrike Topf (Local Organizer), Laetitia Poidevin (ProteoCure Project Manager).

Venue: Hotel Mercure Warszawa Grand https://all.accor.com/hotel/3384/index.en.shtml

The link to the meeting website is: https://proteocure.eu/annual-meeting-2024/

Ligating the Ubiquitin Family: Physiology, Disease, and Future Directions. Bilbao, Jun 24-25, 2024.

https://sebbm.es/actividades-sebbm/2ndsevero-ochoa-conference/



This event is organized by the SEBBM in collaboration with the Fundación Carmen y Severo Ochoa and co-organized by the COST Action ProteoCure. A fantastic lineup of confirmed invited speakers, including Ivan Dikic (GUF), Cristina Mayor-Ruiz (IRB Barcelona), Simona Polo (IFOM), Tim Clausen (IMP), Helen Walden (University of Glasgow), and Jordi Torres-Rosell (IRB Lleida). Selected speakers will complete the programs that includes, poster sessions and short talks. **Registration will remain open until April 30th, with a limited number of spots available. SEBBM members will enjoy a reduced registration fee.**

6th Conference on plant proteases. Stuttgart, September 3-5 2024.

https://plant-proteases-2024.uni-hohenheim.de/

The conference co-organized by ProteoCURE includes presentations from leading experts and newcomers to the field. It aims to promote scientific exchanges highlighting recent advances and inspire the next generation of plant scientist. Our invited speakers includes Simon Stael, Saskia Hogenhout, Nuria Sanchez-Coll, Renier van der Hoorn, Ralf Reski,, Steven Spoel, Marina klemencic and Zach Adam, among others. **Early-bird registration rate until June 15. Standard registration rate until August 31. Limited funds are available to reimburse travel expenses for students and postdocs (students will be served first)**

ProteoCURE Training School: Basics and perspectives of mass spectrometry proteomics. Freiburg, 1-5 of September 2024.

In memorium of Prof. Dr. Ulrich auf dem Keller 1974-2023

https://www.uniklinik-freiburg.de/department-pathologie/gemeinsame-bereiche/lehre/imbs-1.html

Organisers: Oliver Schilling, Oded Kleifeld and Gunnar Dittmar.

This will be a hands-on training school. Mass spectrometry-based proteomics is widely used in many PROTEOCURE research projects. Despite its common usage, many students may find themselves limited by a surface-level understanding of this methodology. Our training class is meticulously designed to bridge this knowledge gap, ensuring participants not only grasp the fundamental concepts but are also abreast with the latest advancements in the domain. With the deluge of data these experiments generate, mastering the subtleties of data interpretation, and understanding the underpinnings of biostatistics is paramount. We aim to make this process intuitive and comprehensive. **Registrations are open**

CONFERENCE GRANTS

ITC conference grants

ProteoCure will support the travels of Shadi Setayeshi and Ebru TURHAL from Instytut Biochemii I Biofizyki Polskiej Akademii Nauk Warszawa - Poland to attend the 7th DNA Polymerases meeting (https://dnapolymerases-warsaw2024.com /). Congratulations to the grantees!

Disseminations conference grants

Carmela Giglione will represent ProteoCure at the FASEB 2024 Protein Lipidation: Enzymology, Signalling, Therapeutics (https://web.cvent.com/event/d933c599-1fac-44ec-a373- a265f25e8bce/summary

Georgia Chachimi will be our ambassador at the HYPOXEU LIVE https://www.hypoxeu.com/dresden-live/

SCIENTIFIC EVENTS REPORTS

41st Winter School on Proteinases and Inhibitors. Tiers, Feb 28 – Mar 3 2024

https://www.plus.ac.at/biosciences/the-department/research-groups/brandstetter/winter-school tiers/?lang=en

The Winter School provided a vibrant platform for researchers in proteolytic enzymes, emphasizing interaction among young scientists and experts across ten sessions. The event fosters global networking and aligns closely with the objectives of fostering innovation in proteolysis research. Additionally, outreach efforts include collaboration with local media for broader dissemination of research findings. Very inspiring, and students were very happy to have the support of ProteoCure. The weather was in favour of science because it was terrible but with magnificent views.

The organization was again very smoothly and efficient by the team of Hans Brandstetter, University of Salzburg, Austria together with Klaudia Brix, Constructor University Bremen. ProteoCure was the co-organizer. The meeting was internationally attractive with more than 80 participants from Austria, Canada, Chile, Czech Republic, Denmark, France, Germany, Poland, Slovenia, UK, and the USA. The main topics were on pathogens, immunity, inhibitors and diagnostics, cancer, substrates and interactome of proteases. The most intensely discussed enzymes were clearly meprin-α and meprin-ß but also new approaches in PROTACs and the proteomics toolbox was extended by the addition of FANTA, CLIPPER 2.0, and PROTOMAP.

Thus, basic, translational, and clinical research was in the focus of this training school that embraced bioinformatics and structural biology as well. The talks were introduced by dedicated Chair Persons and presented in a highly professional manner by young scientists, who demonstrated their interest and talent to dive into academic and industrial careers in support of proteolysis and proteostasis. The high quality and training success resulted in four young investigator awards and twelve recognitions for excellent data presentation. We conclude that the training school supported by this COST continues to be enormously important for the exchanges in ProteoCure

including the practical training entertained by the InhibiTiers. Needless to say, everybody was in a good mood and open to engage in future and ongoing collaborative projects.

We look forward to the next meeting in 2025 – mark your calendars for 12-16 March 2025 to not miss a most informative and very memorable training school on proteases and inhibitors. Klaudia & Hans

PAST, ONGOING AND FUTURE STSM

Simon Tack from the Department of Plant Biotechnology and Bioinformatics Ghent University, Belgium to the Department of Molecular Sciences of the Swedish University of Agricultural Sciences (Dec 1-15, 2023)

Tobias Gökler from the Institute of Applied Synthetic Chemistry TU Wien, Vienna, Austria to the department of Chemistry & Pharmaceutical Sciences of Vrije Universiteit Amsterdam, Netherlands. (Nov 6 to Dec 23, 2023)

Inci Barut from the Pharmacy Department of the Gazi University, Turkey, to the Institute of Neuroscience and Physiology of the University of Gothenburg, Sweden (Nov 16, 2023 – Feb 02, 2024)

Nerea Ruiz from Centre for Research in Agricultural Genomics, Barcelona, Spain to the Institute of Biologie Paris-Seine (IBPS) at the Sorbonne University, France (Jan 01 – Mar 15, 2024)

Ainoa Sanchez Arfelis from the Faculty of Pharmacy and Food Sciences at the University of Barcelona, Spain, to the Centre for Targeted Protein Degradation at the University of Dundee, UK (Jan 15 – Apr 15, 2024)

Oskar Lipiński from the Insitute of Protein biology and Chemistry of Lyon, France to the Oxford Particle Imaging Centre (OPIC) of the University of Oxford, UK. (Feb 10 -Mar 02, 2024, followed by 3 weeks in May 2024)

Fabian Gerth from the Medical School Berlin, Germany to the University College London, UK (March 10 -23, 2024)

Rohit Shrivastava. Centre National de la Recherche Scientifique, Montpellier – France to King's College London, UK (Apr 22 – May 31, 2024).

Rachel Havret from La Rochelle Universite, France to Facultat de Farmàcia i Ciències De l'Alimentació, Barcelona, Spain (May 01 – Jul 31, 2024).

Bojan Ilic from Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia to University of Fribourg, Switzerland (May 5 – Jul 5, 2024).

Sofia Ferreira from Lusófona University - University Centre of Lisbon, Portugal to Utrecht University, Netherlands (May 13 to Jul 12, 2024).

Jitka Vaculíková from The National Centre for Biomolecular Research, Brno, Czech Republic to Max Perutz Labs, Vienna, Austria (May 31 to Jun 28, 2024).

Javier Anton from the Valencia Biomedical Research Fundation, Spain, to the NOVA Medical School, Lisboa, Proteugal (Jun 15 – Jul 15, 2024).

Mostafa Ejtehadifar from Nova Medical School Lisboa, Portugal to Laboratoire de Chimie de Coordination (LCC-CNRS), France (Jun 28 to Jul 27, 2024).

Announcements-Calls

We recently received the excellent news that we will most likely receive a top-up budget for ProteoCure. **Additional ProteoCURE grants will be available** to support travel expenses for our annual meeting in Warsaw, new STSMs, dissemination and ITC conference grants and take in charge expenses of additional trainers/trainees for the Proteomics training school organised in Freiburg, September 2024. **In the case we confirm this additional funding we will inform you and open new calls in our ProteoCURE web page.**

In addition, **ProteoCure travel grants** are available to attend the Plant Proteases 2024 Meeting in Stuttgart.

PUBLISH RELEVANT INFORMATION IN OUR WEB PAGE OR NEWSLETTER IS EASY.

Send us an email to: issemination@proteocure.eu

WEBINARS

Most webinars will take place every second Thursdays, 1PM, CET. Links will be sent by email or posted in our web page. Save the dates:

Stefan Knapp. Apr 18, 13:00 – 14:00 CEST
Development and characterization of chemical probes targeting the ubiquitin system

Christopher M Overall. Apr 22, 11:00 – 12:00 CET
Protein TAILS tell remarkable Tales: Purification of original and neo-protein termini reveals pervasive proteolytic processing in proteomes

Shalini Padmanabhan. May 23, 13:00-14:00 CET.
The Michael J. Fox Foundation's Research Strategy

Frederic Frottin. Jun 06, 13:00-14:00 CET. A good body for nuclear proteostasis

Michael Rape. Jul 11, 13:00-14:00 CET. Title to be announced.

INTERVIEWS WITH YOUNG SCIENTIST THAT OBTAINED A STSM FELLOWSHIP

Dear colleagues first of all thanks for accepting to answer our questions. The idea of this interview is to obtain feedback from you about the STSM experience you had. Please answer our following questions:

1. What were your activities during your STSM?
2. What impact the STSM had in your carrier?
3. Include a short description of the out puts of this STSM

Chrysa Filippopoulou

Institution: Georgia Chachami group, Lab. Of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa Greece

Hosting institution: Markus Bohnsack group, University Medical Center Göttingen, Dept. of Molecular Biology, Gottingen, Germany

Dates of the STSM: 12 June 2022-5 July 2022

Q1. I received an STSM fellowship in 2022 as a PhD student in Dr. Georgia Chachami's group at the University of Thessaly, Greece, specializing in the SUMOylation of proteins and their impact on the cellular response to hypoxia. My main doctoral project focused on investigating the role of EXOSC10, an exoribonuclease involved in ribosome biogenesis and RNA turnover, and its SUMOylation during hypoxia. During my STSM, I had the privilege of visiting our collaborators, the Bohnsack Group at the Department of Molecular Biology, University Medical Center Göttingen, Germany. There, I engaged in cutting-edge RNA biology research hands-on. During my time there, I actively participated in experiments and received training in methods related to rRNA processing, such as Northern blotting and in vitro exoribonuclease assays. Additionally, I gained experience in transcriptome-wide analysis and bioinformatics.

Q2. During my STSM, I had the chance to generate crucial data for my PhD project, resulting later in a publication (Filippopoulou C, et al. Hypoxia-driven deSUMOylation of EXOSC10 promotes adaptive changes in the transcriptome profile. Cell Mol Life Sci. 2024;81(1):58). This experience allowed me to not only view my PhD project from the perspective of an RNA specialist but also to broaden my scientific horizons and gain hands-on experience with state-of-the-art RNA-related techniques, enriching my skillset. Additionally, working in a lab abroad provided me with the chance to meet and connect with other young and senior scientists, fostering valuable networking opportunities. The STSM also had a profound effect on my professional development, enriching my CV and highlighting my ability to thrive in new and collaborative environments. Overall, the STSM not only accelerated my research progress per se but also facilitated my personal and professional maturity, allowing me to broaden my scientific expertise and pursue future fellowship opportunities with confidence.

Q3. To understand the dynamics of SUMOylation in hypoxia, we conducted immunoprecipitation experiments of all endogenously SUMOylated proteins. We discovered that hypoxia strongly decreases the SUMOylation of Exosome

subunit 10 (EXOSC10), the catalytic subunit of the RNA exosome, in an HIF-independent manner. Functionally, EXOSC10 is involved in ribosome biogenesis, RNA turnover, surveillance, and processing of various RNA species. Our study demonstrated that ubiquitin-specific protease 36 (USP36) promotes SUMOylation of EXOSC10, while SUMO1/sentrin-specific peptidase 3 (SENP3) mediates its deSUMOylation. To comprehensively study the effects of hypoxia on this mechanism, we employed advanced techniques including confocal microscopy and immunoprecipitation experiments. These experiments revealed that under hypoxia, EXOSC10 dissociates from USP36 and translocates from the nucleolus to the nucleoplasm concomitant with its deSUMOylation. Loss of EXOSC10 SUMOylation did not significantly impact rRNA maturation but altered the mRNA transcriptome by regulating the expression levels of hypoxia-related genes. Overall, our publication suggests that dynamic modulation of EXOSC10 SUMOylation and localization in hypoxia regulates the RNA degradation machinery to facilitate cellular adaptation to low oxygen conditions.

Roger Castaño

Institution: Carles Galdeano group, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Barcelona Spain

Hosting institution: Brenda Schulman group, Max Planck Institute of Biochemistry, Martinsried Germany

Dates of the STSM: 21/11/2022-30-01-2023

Q1. A former PhD student in Galdeano's Lab initiated a fragment-based approach to discover binders of Fbxw7, resulting in the identification of 9 confirmed binders. The objective of the brief visit to Brenda Schulman's lab was to determine the structure of Fbxw7 bound to the confirmed fragments obtained from the fragment-based approach. Fbxw7 without the dimerization domain, in complex with Skp1, was expressed. Expression was carried out in E. coli cells using a pGEX vector capable of expressing two proteins as a dicistronic message. Despite obtaining a low yield of protein (0.3 mg/L of culture), several attempts were made to increase the yield. Insect cell expression was attempted to increase yield. The two genes were subcloned into a pACEBacDual plasmid, changing the affinity tag to Twin-strepTag but leaving the original sequence intact. Expression was achieved by infecting Hi5 insect cells with the P3 viral stock produced using Sf9 cells. To assess the quality of the complexes obtained with both systems, we conducted size exclusion chromatography, SDS-PAGE, and mass spectrometry analyses. Additionally, a proteomics analysis was conducted to confirm that insect cells did not introduce any new post-translational modifications that could affect the behavior of the protein complex during crystallization. Published conditions (Bing Hao et al., Molecular Cell, 2007) were attempted, but they were unsuitable for co-crystallization with the DMSO used to solubilize the compounds. A screening campaign was initiated to discover new conditions compatible with DMSO and small molecules. These conditions were further tested with different salts and PEGs screens, but the resulting crystals were too small for measurement. We conducted several rounds of optimization to improve crystal size and shape, testing various reagent concentrations, drop sizes, drop ratios, buffer systems, and temperatures (10 and 18°C) to slow down the nucleation process.

Q2. On a personal level, collaborating with Prof. Schulman opened the doors for me to become acquainted with her laboratory and its members, allowing me to engage with excellent science. Leveraging their expertise and facilities significantly accelerated the project, yielding invaluable data for my thesis. Additionally, thanks to the STSM, I had the opportunity to travel and become acquainted with the scientific community in the fields of ubiquitin and targeted protein degradation.

Q3. The Short-Term Scientific Mission has contributed to achieving the research coordination objectives outlined in the memorandum of understanding of the Proteocure Action. Specifically, it has facilitated the fulfillment of objectives 1 and 2 by fostering scientific connections within the proteolysis community and sharing expertise in drug discovery and structural biology. The collaboration between the Schulman's and Galdeano's labs has forged a new bond, laying the groundwork for future collaborations and joint projects of common interest. The expertise in E3 ligases and structural biology from Schulman's lab has complemented our expertise in drug discovery, enhancing our understanding of the interactions between PROTACs and CRL complexes.

Corentin Bouvier

Institution: Manuel S. Rodriguez's team: Dendrimers and heterochemistry. Laboratory of Chemistry of Coordination-CNRS.

Hosting institution: Carles Galdeano group, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Barcelona Spain.

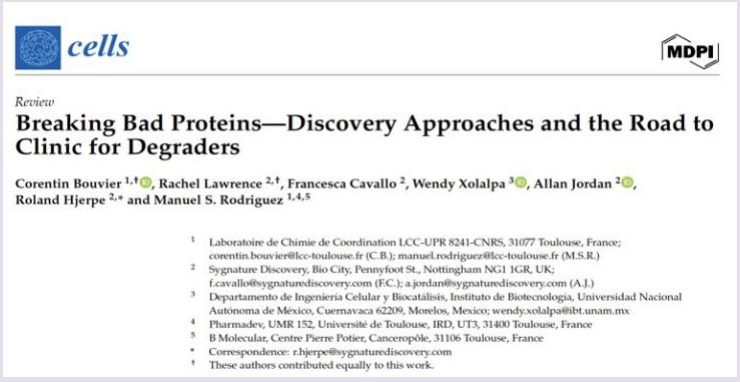
Dates of the STSM: 4th April, 2022 to 1st July 2022

Q1. My doctoral project focuses on the resistance to the proteasome inhibitor Bortezomib (BTZ). This inhibitor was approved in the early 2000s for the treatment of mantle cell lymphoma (MCL). As the emergence of resistance in patients limits BTZ's therapeutic potential, it is crucial to develop new combination therapies with the ability to overcome drug refractoriness. Our team demonstrated that resistance to the proteasome inhibitor BTZ permanently activates its degradation by a mechanism of selective autophagy called proteaphagy. By genetically silencing the proteaphagy receptor p62, we found that intervening in proteaphagy leads to effective apoptosis in BTZ-resistant cells. My primary objective is to develop innovative molecules aiming to target the proteaphagy receptor p62 in MCL cells. Our focus is particularly on developing (macro)molecular multivalent platforms that encompass proteolysis targeting chimeras (PROTACs) as well as highly ordered, branched polymeric structures known as dendrimers. The specific aim of my STSM was to conduct computational molecular modeling studies to elucidate the binding site and binding mode of p62 ligands. We focus on the first p62 inhibitor documented in the literature: XRK3F2. We used pivotal information during the STSM to optimize a ligand, which will then be synthesized upon returning to the home laboratory and used for the development of multivalent platforms. To achieve this, we initially identified druggable surfaces on the p62-ZZ protein structure (PDB 6MJ7). The hosting laboratory developed a methodology known as MDMix, which identifies new binding sites and predicts their druggability. MDMix provided detailed molecular information (pharmacophores) about interaction preferences, which was extremely helpful in discovering which regions of the protein are more likely to be targeted. Once the druggable sites were identified, docking studies using rDock were performed to identify putative binding modes of XRK3F2.

Q2. Through collaboration with experts in computational chemistry, I acquired cutting-edge computational techniques and methodologies, thus enhancing my research capabilities. This understanding has improved my ability to interpret experimental results effectively and design experiments with precision. Joining a research group with extensive experience in targeted protein degradation further deepened my understanding of this field. Moreover, the STSM provided invaluable opportunities to collaborate with researchers from diverse institutions and backgrounds, facilitating the strengthening of partnerships between my home and host institutions. The STSM profoundly impacted my professional development, enriching my CV by fostering the development of new skills and showcasing my adaptability to new environments.

Q3. As p62 has not been fully crystallized, simulations were conducted only on a specific domain of p62. Unfortunately, this model did not allow us to observe the stability of the hypothesized binding modes through molecular dynamic simulations. However, during my STSM, I successfully identified a ligand derived from XRK3F2, which was concomitantly reported in the literature. The ligand, named YOK-2204, demonstrated a higher affinity for the target, as it fulfilled more hotspots compared to XRK3F2. Additionally, unlike XRK3F2, YOK-2204 possesses a solvent-exposed reactive part, rendering it more suitable for synthesis within multivalent platforms. The findings from this STSM are crucial for the ongoing development of novel molecules, which are currently undergoing synthesis.

PUBLICATIONS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MDPI

Review

Breaking Bad Proteins—Discovery Approaches and the Road to Clinic for Degraders

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SUMOylation controls Hu antigen R posttranscriptional activity in liver cancer

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