Memoirs of the Scientific Sections of the Romanian Academy
Tome XLVIII, 2025

BIOLOGY

ENZYMES, IDEAS AND PEOPLE: THE DEPARTMENT OF ENZYMOLOGY OF THE INSTITUTE OF BIOCHEMISTRY OF THE ROMANIAN ACADEMY

OTILIA CRISTINA DONȚU[1] AND ȘTEFAN-EUGEN SZEDLACSEK[1]

[1] *The Institute of Biochemistry, Romanian Academy, Bucharest, Romania*
Corresponding author: szedlacs@yahoo.co.uk

This paper outlines the history of the Department of Enzymology at the Institute of Biochemistry of the Romanian Academy. A leading centre in Romania, the department is recognised for its long-standing contributions to the study of enzymes and their mechanisms of action. Founded in early 1991, it specialises in the structure-function relationships of signaling enzymes, with a particular focus on protein tyrosine phosphatases. Over the years, the department has trained numerous undergraduate and doctoral researchers who now work in prestigious institutions across Europe and the United States. Drawing on expertise in molecular biology, crystallography, and enzyme kinetics, it conducts innovative research in areas such as cell signaling, tumour biology, acute myeloid leukaemia (AML), neurodegenerative diseases, and biotechnological applications. The department's international visibility is strengthened through collaborations that have resulted in publications in high-impact journals, patents, and joint projects with renowned research centres worldwide.

Keywords: enzymology, Romanian Academy, protein tyrosine phosphatases, cell signaling, biochemical applications.

1. INTRODUCTION

Enzymes are essential biological macromolecules that catalyse nearly all chemical reactions in living organisms. By lowering the activation energy of specific reactions, they ensure the proper functioning of complex metabolic and signaling networks. Structurally, enzymes are mostly globular proteins whose three-dimensional conformation defines their catalytic activity and substrate specificity. Their efficiency and selectivity make them key regulators of vital processes such as cellular metabolism, DNA replication and repair, signal transduction, and programmed cell death. Owing to these properties, enzymes have also become indispensable in biotechnology, medicine, and industry, being applied in drug development, diagnostics, environmental protection, and the synthesis of

biofuels and biodegradable materials. In this context, the Department of Enzymology of the Institute of Biochemistry of the Romanian Academy has played an important role in advancing enzymology in Romania. Established in early 1991, the department built a strong tradition of research on the structure-function relationships of signaling enzymes, particularly protein tyrosine phosphatases, combining biochemical, structural, and cellular approaches to elucidate their physiological and pathological roles.

2. FOUNDING AND HISTORICAL DEVELOPMENT

The Department of Enzymology of the Institute of Biochemistry of the Romanian Academy (IBAR) was formally established in early 1991, although it had been functioning informally since 1990, under the coordination of Dr. Ștefan E. Szedlacsek, who at that time was conducting his postdoctoral research at the University of Illinois at Urbana-Champaign (USA), in the laboratory of Professor Fred A. Kummerow.

Professor Fred Kummerow was a pioneering biochemist, internationally recognized as the discoverer of trans fats and among the first to link dietary lipids to cardiovascular disease. Already in 1957, he published in Science the first paper suggesting this causal connection - initially rejected by industry and even parts of the scientific world. He later demonstrated that oxidized cholesterol (oxysterols), rather than native cholesterol, plays a central role in atherogenesis, fundamentally reshaping the biochemical understanding of lipid-mediated vascular damage. A tireless public health advocate, he ultimately compelled the U.S. FDA to ban artificial trans fats in 2015, declaring: “Science won out.” During his postdoctoral fellowship in Kummerow’s laboratory, Dr. Szedlacsek contributed directly to this oxysterol research direction, co-authoring a study published in Journal of Biological Chemistry [1] that elucidated the esterification of oxysterols by human plasma lecithin-cholesterol acyltransferase (LCAT) – providing one of the earliest mechanistic insights into how the body detoxifies oxidized lipids. The creation of the Department of Enzymology represented both an institutional and a scientific milestone. The new department crystallized a decade of kinetic and theoretical studies that had already placed Romanian enzymology in dialogue with major international centres. During the 1980s, several pioneering works had laid the conceptual foundations that would later define the department’s research identity. Among these were studies on isoenzyme activity determination [2], early analyses of tight-binding inhibition mechanisms [3], and collaborations on enzyme cascade regulation [4]. These pioneering works were carried out in collaboration with leading figures in the field of enzyme kinetics in the late twentieth century, including Athel Cornish-Bowden and, later, Ronald G. Duggleby. Their theoretical contributions had a significant impact on the development of modern enzymology.

Figure 1. Professor Kummerow in his laboratory at the University of Illinois at Urbana-Champaign (1991–1992).

Figure 2. Dr. Ştefan Szedlacsek during his postdoctoral research in the laboratory of Professor Kummerow, University of Illinois at Urbana-Champaign (1991–1992).

The turning point came with the publication of the seminal paper "Enzyme catalysis as a chain reaction" in The Biochemical Journal (1991) [5]. This study, authored by Ştefan E. Szedlacsek, Ronald G. Duggleby, and Marcel O. Vlad, introduced a new kinetic mechanism in which enzymatic catalysis was interpreted as a chain reaction, a concept that extended beyond the classical Michaelis- Menten framework. Importantly, this paper was the first to list the affiliation "Department of Enzymology, Institute of Biochemistry, Bucharest, Romania", thus marking the department's formal presence in the international scientific literature. Following its official establishment in 1991, the Department of Enzymology of the Institute of Biochemistry of the Romanian Academy (IBAR) quickly gained recognition for its intellectual creativity and for its collaborations with leading institutions such as the University of Queensland (Australia), the Centre de Biochimie et de Biologie Moléculaire (CNRS-Marseille, France), and several Romanian universities and research institutes. The conceptual advances initiated by the 1991 paper continued to influence later studies on signal transduction, redox regulation, and the mathematical modelling of enzymatic systems, which became defining pillars of the department's long-term scientific identity.

3. FROM THEORETICAL MODELLING TO EXPERIMENTAL ENZYMOLOGY: A DECADE OF DISCOVERY AND INNOVATION (1991 – 2001)

The decade following the foundation of the Department of Enzymology marked a progressive shift from theoretical models to experimental and structural approaches, leading to several pioneering achievements that positioned the group at the forefront of biochemical research in Romania. Early studies provided mathematical and kinetic frameworks for understanding enzyme cascades and signal

amplification in interconvertible systems [4, 6]. These works represented some of the first Eastern European contributions to quantitative enzymology and laid the foundation for subsequent modelling of biological regulation. The Department expanded its investigations to the kinetic behaviour of complex catalytic and inhibitory systems. Theoretical analyses of slow- and tight-binding inhibition [7] established methodological standards for the evaluation of enzyme–inhibitor interactions, later used in pharmacological studies. At the same time, collaborative efforts in the area of lipid metabolism strengthened the conceptual link between enzymology and biomedical chemistry. Another important contribution of this decade described the pH-dependent hysteretic behaviour of human myeloblastin, a leukocyte protease exhibiting slow conformational transitions that affect catalytic activity [8]. This study provided one of the earliest kinetic explanations for conformational hysteresis in human enzymes. In the same period, non-steady-state models of metabolic regulation were proposed, showing how enzymes dynamically respond to external effectors and time-dependent stimuli [9]. After 1994, the Department developed molecular cloning and recombinant protein expression, performing the first successful cloning experiments in Romania. Using these techniques, the team expressed and characterized a couple of receptor-like protein tyrosine phosphatases, including RPTPμ [10]. These studies enabled a gradual transition from the exclusive analysis of kinetic properties to the investigation of structure–function relationships, by correlating biochemical data with insights obtained from structural analyses. The structural and kinetic analyses conducted during this period revealed how the catalytic domains within these phosphatases regulate each other and paved the way for subsequent crystallographic studies that would later elucidate the molecular mechanisms of MAP kinase regulation. Specifically, building on these advances, the team led by Dr. Ștefan E. Szedlacsek subsequently expanded its experimental approaches to explore phosphatase-MAP kinase interactions and the mechanisms by which these enzymes control intracellular signaling fluxes [10,11]. These achievements were the result of close collaboration between Dr. Ștefan E. Szedlacsek and a group of enthusiastic young scientists: Radu Aricescu, Tudor Fulga, and Valeriu Bogdan Cișmașiu, who combined theoretical insight with advanced molecular biology and biochemical experimentation. Their cohesion and determination often evoked the spirit of *"the four musketeers* of enzymology", united by a shared vision of scientific excellence and mutual trust.

Through perseverance, creativity, and teamwork, they transformed the Department of Enzymology into a centre of innovation and international visibility, proving that high-level research could flourish even in modest conditions. During this decade, through the initiative of Dr. Ștefan E. Szedlacsek, the Department of Enzymology became the first unit within the Institute of Biochemistry to benefit from prestigious international fellowships and research grants, including those awarded by the Alexander von Humboldt Foundation, the Deutsche Forschungsgemeinschaft (DFG), and the NATO Linkage Grant Program. These fellowships enabled extended research stays in leading German institutions such as the Institute of Biochemistry, Christian

Albrechts University of Kiel (1992–1993), the Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, Frankfurt am Main (1996–1997), and the Max Planck Institute of Molecular Physiology, Dortmund (1998–2002).

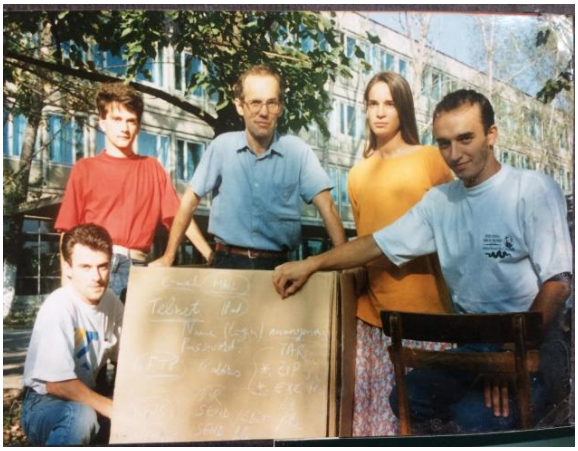
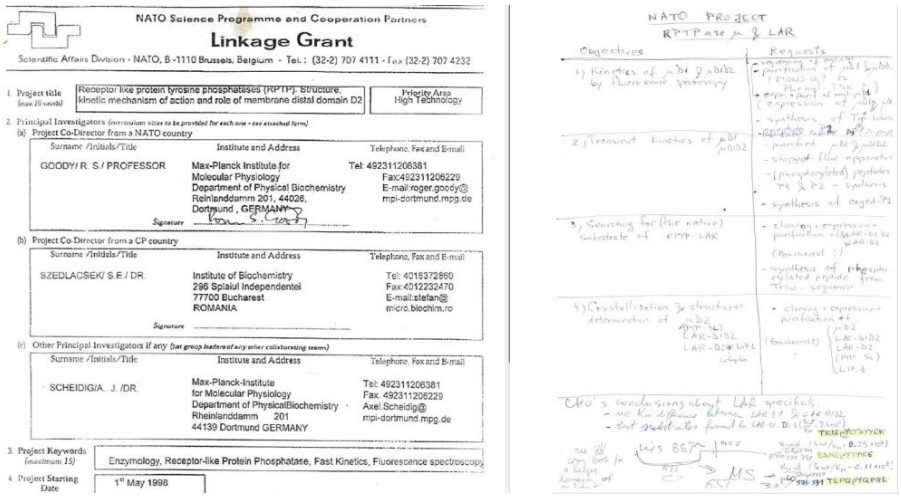


Figure 3. The team that contributed to the first molecular cloning and recombinant protein expression experiments in Romania, within the Department of Enzymology of the Institute of Biochemistry of the Romanian Academy. From left to right: Valeriu Bogdan Cişmaşiu, Radu Aricescu, Ştefan Szedlacsek, Ana-Maria Aricescu and Tudor Fulga.



NATO Science Programme and Cooperation Partners

Linkage Grant

Scientific Affairs Division - NATO, B -1110 Brussels, Belgium - Tel.: (32-2) 707 4111 - Fax (32-2) 707 4232

1. Project title (max 10 words): Receptor like protein tyrosine phosphatases (RPTP). Structure, kinetic mechanism of action and role of membrane distal domain D2 — Priority Area: High Technology

2. Principal Investigators (curriculum vitae to be provided for each one - see attached form)

(a) Project Co-Director from a NATO country

Surname /Initials/Title	Institute and Address	Telephone, Fax and E-mail
GOODY/ R. S./ PROFESSOR	Max-Planck Institute for Molecular Physiology Department of Physical Biochemistry Reinlanddamm 201, 44026, Dortmund , GERMANY	Tel: 492311206381 Fax:492311206229 E-mail:roger.goody@ mpi-dortmund.mpg.de

Signature

(b) Project Co-Director from a CP country

Surname /Initials/Title	Institute and Address	Telephone, Fax and E-mail
SZEDLACSEK/ S.E./ DR.	Institute of Biochemistry 296 Splaiul Independentei 77700 Bucharest ROMANIA	Tel: 4018372890 Fax:4012232470 E-mail:stefan@ micro.biochim.ro

Signature

(c) Other Principal Investigators if any (list group leaders of any other collaborating teams)

Surname /Initials/Title	Institute and Address	Telephone, Fax and E-mail
SCHEIDIG/A. J. /DR.	Max-Planck-Institute for Molecular Physiology Department of PhysicalBiochemistry Rheinlanddamm 201 44139 Dortmund GERMANY	Tel: 492311206381 Fax. 492311206229 Axel.Scheidig@ mpi-dortmund.mpg.de

3. Project Keywords (maximum 15): Enzymology, Receptor-like Protein Phosphatase, Fast Kinetics, Fluorescence spectroscopy

4. Project Starting Date: 1st May 1998

NATO PROJECT

RPTPase μ & LAR

Objectives — Requests

1) Kinetics of μD1 & μD1D2 by fluorescence spectroscopy

- purification of μD1 & μD1D2
- expression of μD1 & μD1D2

2) Treatment kinetics of μD1 / μD1D2

- synthesis of (phosphorylated) peptides

3) Searching for (the native) substrate of RPTP-LAR

- cloning + expression + purification of LAR-D1D2

4) Crystallization & structure determination of μD2, LAR-D1D2, LAR-D2#, LAR

- cloning + expression + purification of μD2, LAR-D1D2, LAR-D2, LAR

CNRS's conclusions about LAR specificity

- our kinetic data
- but substrates found in LAR or RPTPμ

Building upon these collaborations, several young researchers from the Department also benefited from advanced training in partner laboratories, where they gained experience in protein purification, crystallization, and kinetic analyses. Beyond these scientific milestones, the late 1990s also represented a formative period for the Department's internal scientific culture. Regular seminars, collaborative discussions, and the mentoring of undergraduate and doctoral students fostered an atmosphere of intellectual exchange and creativity. Despite limited resources, the group's ability to generate original ideas and uphold rigorous scientific standards became a model for other emerging research units in Romania. The Department's early openness toward international dialogue not only enhanced its visibility but also laid the foundation for a sustainable tradition of academic excellence and mentorship.

Figure 5. Evolution of Enzymology Research (1990–2001).
From Theoretical Models to Molecular Insights.

Figure 6. Dr. Ştefan Szedlacsek during his Humboldt Fellowship at the Institute of Biochemistry, Christian Albrechts University of Kiel (1993), a period that marked the beginning of his long-term scientific collaborations in Germany.

Figure 7. Dr. Ştefan Szedlacsek in the laboratory of the Max Planck Institute of Biophysics, Frankfurt am Main (1996), during his Humboldt Fellowship, a period that consolidated his scientific collaborations in the field of molecular enzymology.

As a direct consequence of this growing international visibility including the NATO Linkage Grant mentioned above, the Institute of Biochemistry of the Romanian Academy (IBAR) became the first Romanian research institution to

implement an international peer-reviewing program funded by the NATO Scientific Division, under the auspices of the Romanian Academy, with a follow-up call in 2000. This initiative placed the Department of Enzymology at the forefront of scientific innovation and evaluation in Romania, marking the transition from a developing research group to a fully integrated actor within the international biochemical community.

4. BEYOND CRYSTALS: EXPANSION INTO MOLECULAR BIOLOGY AND BIOMEDICAL APPLICATIONS (2001–2010)

The beginning of the new millennium brought a decisive transformation within the Department of Enzymology, marked by the transition from pioneering kinetic and theoretical studies toward fully fledged structural and molecular enzymology. If the 1990s represented the ascent from theory to experiment, the decade between 2001 and 2010 became the era of structure and mechanism, a period defined by the first crystallographic determinations performed in Romania and by the growing integration of three-dimensional insight with cellular signaling and human disease models. One of the most significant achievements marking this transition was the crystallographic characterization of the PTP-SL/PTPBR7 catalytic domain [12], obtained in collaboration with Professor Axel Scheidig (University of Kiel, Germany). Beyond its structural importance, this work symbolized a shift in the Department's scientific vision, from the elucidation of enzyme mechanisms to their roles in cellular and disease contexts.

Complementary theoretical and experimental work, conducted in collaboration with John Ross and Marcel Vlad (Stanford University), extended the department's expertise toward nonlinear kinetics and generalized Fisher relations, reinforcing its impact on molecular enzymology and systems biology [13].

Further investigations were performed during Dr. Szedlacsek's Humboldt research fellowship at the Max Planck Institute of Molecular Physiology (Dortmund), representing an international extension of the department's research activity. Thus, in collaboration with Roger S. Goody and Kirill Alexandrov, fluorescent analogues of isoprenoids were developed, enabling for the first time non-radioactive profiling of prenyltransferase inhibitors and the visualization of isoprenoid incorporation into mammalian cells [14].

During the same period, the group mapped the substrate specificity of protein tyrosine phosphatases, showing that these enzymes recognize defined peptide motifs, a paradigm shift with direct implications for the design of selective inhibitors [15].

Research directions also expanded toward tumor signaling, including studies on PRL-3, a phosphatase associated with metastatic processes, and toward structural biology of catalytic domains expressed and purified in Bucharest, where preliminary crystallographic data were reported [16]. A particularly important

milestone for the Department during this decade was the collaboration with Nobel laureate Hartmut Michel, which led to the demonstration that the MAM (meprin/A5-protein/PTPμ) domain functions as a homophilic binding interface promoting the lateral dimerisation of receptor-like phosphatase μ [17]. This study offered a crucial mechanistic insight into receptor organization at the cell membrane and marked a defining moment in the consolidation of structural enzymology within our Department.

The European dimension of the Department's activity was further consolidated through participation in the PTP-NET Marie Curie Research Training Network (FP6, 2007–2011), dedicated to the study of protein tyrosine phosphatases. This program facilitated scientific exchanges and international training, including the supervision of Early Stage Researcher (ESR) Sujay Turuvekere Mallikarjuna from India, who later completed his PhD under the supervision of Professor Szedlacsek. His work focused on the structural and functional analysis of phosphatases, contributing to the Department's international visibility.

Figure 8. The Romanian team that participated in the PTP-NET Marie Curie Research Training Network. (FP6, 2007–2011).
From left to right: PhD student: Georgiana Petrăreanu, Postdoctoral Researcher **Mihaela Bălașu**$^{+}$, Technical assistant Adina Monea, PhD students: Mihaela Pascaru and Rodica Badea, and Professor Dr. Ștefan Szedlacsek.

The consortium focused on protein tyrosine phosphatases (PTPases), integrating structural, kinetic and molecular approaches.

Marie Curie Research Training PTP-NET (FP6)

- Enzymatic characterization and identification of the native substrate for a non-classical PTPase
- Molecular optimization of a xylose-metabolizing enzyme
- Mathematical analysis of response experiments in chemical and biochemical kinetics.

This conceptual synthesis culminated in an invited review published in The FEBS Journal (2008), co-authored with Lydia Tabernero and Yvonne Jones, summarizing the structural and functional relationships within the protein tyrosine phosphatase superfamily [18].

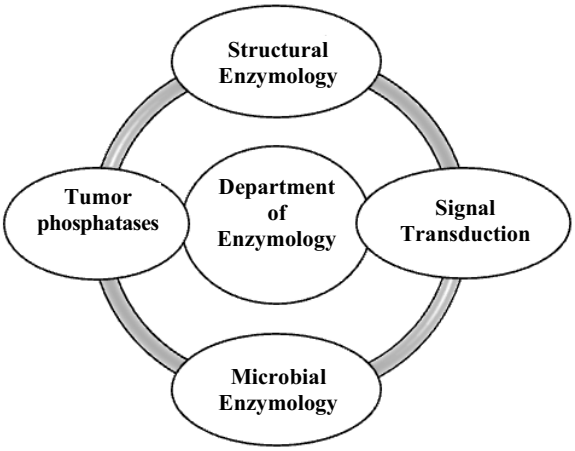


Figure 9. Conceptual representation of the main research directions of the Department of Enzymology (2001–2010).

Another generation of talented researchers continued to strengthen the Department's scientific profile through advanced studies aimed at elucidating the structure and function of essential proteins using modern X-ray diffraction and super-resolution microscopy techniques. They contributed to the understanding of the enzymatic properties and substrate specificity of phosphoketolases from various bacterial species, providing biochemical and kinetic data of biotechnological relevance. The decade concluded with crystallographic analysis of D-xylulose-5-phosphate phosphoketolase from *Lactococcus lactis* [19], marking the Department's expansion into microbial and industrial enzymology. During this period, the Department also contributed decisively to the establishment of DIPROXAL, the first national facility for protein crystallography within the Institute of Biochemistry of the Romanian Academy, and the first of its kind in Romania, dedicated to the three-dimensional structural determination of proteins. This initiative opened a new stage in structural biology and consolidated the Department's role as a leading center of excellence in

molecular and structural research. Driven by intellectual ambition, scientific discipline, and a strong spirit of teamwork, this generation of scientists carried forward the intellectual legacy and vision of the Department of Enzymology, transforming it into a reference point for modern enzymology. Looking back, the 2000s were not only a decade of scientific progress but also a decade of intellectual courage and professional camaraderie, where behind every article, crystal structure, and catalytic assay stood people united by a common conviction: that Romanian science can create genuine value and inspire future generations.

5. ENZYMES, SIGNALING, AND NEURODEGENERATIVE DISEASES: NEW DIRECTIONS IN THE RESEARCH OF THE DEPARTMENT OF ENZYMOLOGY (2011–2021)

The decade between 2011 and 2021 marked a stage of scientific maturity for the Department of Enzymology. It was characterized by thematic diversification and a gradual transition from the study of isolated catalytic mechanisms toward the integration of enzymes within complex signaling and pathophysiological networks. The research activity of the Department continued to focus on protein tyrosine phosphatases (PTPs), which are essential regulators of cellular homeostasis and signal transduction [20]. Significant methodological progress was achieved through the refinement of expression, purification, and kinetic analysis protocols for representative phosphatases such as RPTPμ, EYA3, and PTPBR7. These advances provided reproducible experimental systems for comparative activity studies.

A major research direction focused on EYA3, an atypical dual-function phosphatase with both catalytic and transcriptional co-activator roles. Studies demonstrated that EYA3 is a substrate of the Src kinase, and that its phosphorylation state controls both subcellular localization and actin cytoskeleton remodeling through interaction with WDR1, a newly identified substrate [21]. Subsequent mass-spectrometric analyses identified three key phosphotyrosine residues (Y77, Y96, and Y237) involved in these processes. These findings established a connection between the post-translational regulation of EYA3, cell proliferation, and cytoskeletal dynamics [22]. In parallel with signaling-oriented studies, structural enzymology advanced toward the characterization of thiamine pyrophosphate (TPP)-dependent phosphoketolases. This is a distinct enzyme family essential for microbial carbohydrate metabolism. Early biochemical investigations elucidated the enzymatic properties and substrate specificity of phosphoketolases from several bacterial species, including *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Pseudomonas aeruginosa* [23]. Later, the crystal structure of D-xylulose-5-phosphate phosphoketolase (XPK) from *Lactococcus lactis* was determined. This represented the first structural model of this enzyme worldwide and provided detailed insight into substrate binding and active-site

organization [24]. This work consolidated the Department's expertise in microbial and structural enzymology.

In collaboration with Professor Alexandru Babeş from the University of Bucharest, the Department explored the interplay between kinase and phosphatase signaling in the regulation of ion channels. This joint work demonstrated that the TRPM8 cold receptor channel undergoes Src-mediated tyrosine phosphorylation, a modification that modulates both neuronal excitability and tumor progression. These findings highlighted the complex interdependence between enzymatic signaling and sensory transduction [25]. Toward the end of the decade, the Department's research evolved toward neuroenzymology. Investigations focused on enzymatic mechanisms underlying cognitive decline and neurodegenerative diseases. A major conceptual breakthrough emerged within the Department from the hypothesis that the phosphatase STEP (STriatal-Enriched tyrosine Phosphatase) could be selectively inhibited by short peptides designed to block its interaction with neuronal receptor.

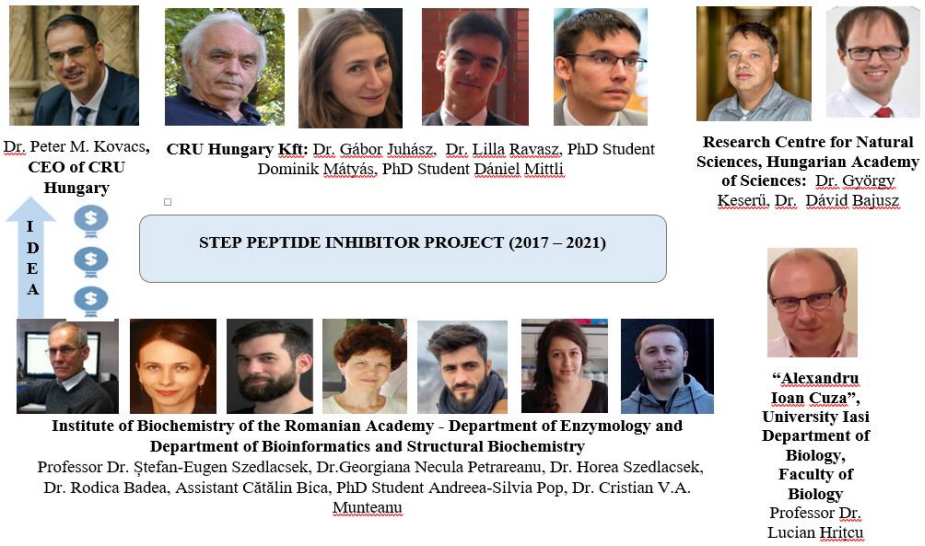


Figure 10. Schematic representation of the collaborative network established around the STEP project. The project originated from an idea proposed by Professor Szedlacsek and was supported by a private grant from Dr. Peter M. Kovács (CEO of CRU Hungary). The funding enabled a bilateral collaboration between.

This innovative concept attracted the interest of a Hungarian private investor and led to a Romanian – Hungarian collaboration focused on rational peptide design and preclinical testing.

The partnership resulted in the development of inhibitory peptides that selectively target the interaction between STEP and the AMPA receptor subunit GluA2, thereby modulating synaptic signaling and cognitive processes [26]. Because the overactivation of STEP contributes to synaptic dysfunction and memory loss in Alzheimer's disease, this project opened new perspectives for enzyme-based therapeutic strategies in neurodegenerative research. Through molecular modeling and biochemical assays, the teams identified several short peptides capable of blocking this interaction. These molecules restored normal synaptic activity and, in animal studies, improved memory performance in rats with induced cognitive impairment. This achievement represents a bridge between enzymology and neurobiology, demonstrating how fundamental research can inspire therapeutic strategies for brain protection and memory recovery. These advances were further consolidated by the U.S. Patent US11834518B2 (2023), which formally recognized the therapeutic potential of STEP phosphatase as a molecular target [27].

During this period, the Department also underwent a generational renewal, marked by the arrival of several young and enthusiastic researchers who brought new energy and perspectives to ongoing projects. In 2019, Cătălin Bica joined the Department as a research assistant, followed by PhD students Adina Puiu and Andrei Vasilescu in 2020, and PhD student Andra Coșoreanu in 2021. Their involvement contributed to broadening the Department's methodological and thematic range, ensuring continuity between established expertise and emerging scientific directions.

These achievements also marked the culmination of a period defined by conceptual integration and international cooperation. The groundwork laid during 2011–2021 later expanded into new collaborative frameworks. One of them was a bilateral research program (2018–2021) between the Romanian Academy and the Hungarian Academy of Sciences, which further diversified the Department's expertise toward molecular imaging and the development of radiolabeled affibody molecules for biomedical applications.

6. FROM MOLECULAR MECHANISMS TO TRANSLATIONAL ENZYMOLOGY: A NEW PARADIGM DRIVEN BY A YOUNG RESEARCH TEAM (2022 – PRESENT).

In recent years, the research activity within the Department of Enzymology has entered a new and dynamic phase, marked by a shift in focus from studying isolated enzymatic mechanisms toward exploring how such knowledge can be transformed into tangible therapeutic and diagnostic solutions. This transformation took shape through new international collaborations and an increasing openness toward applied research directions, bridging fundamental biochemistry with translational medicine. A key milestone in this evolution was the Fulbright

Research Grant (2022–2023), carried out at the MD Anderson Cancer Center (University of Texas, Department of Leukemia), one of the world's leading oncology research institutions. Within this program, the conceptual basis was established for an ambitious line of investigation focused on the use of affibodies, small engineered proteins capable of recognizing specific molecular targets on tumor cells, as molecular vehicles for the controlled delivery of antileukemic drugs.

This initiative built upon the Department's expertise in protein design and purification and opened the way toward translational approaches that combined enzymology with molecular targeting. A first major outcome was the development of novel Mn(II) – based diagnostic agents, continuing the Romanian-Hungarian bilateral collaboration. These studies demonstrated the feasibility of using Mn-based isotopes for dual PET/MRI imaging and introduced a new class of safe and efficient diagnostic probes. This line of research culminated in the synthesis and characterization of innovative [52Mn]-labeled affibody radiotracers capable of selectively targeting HER2-positive tumors [28, 29].

Figure 11. The present research team of the Department of Enzymology, representing the new generation of researchers continuing the Department's tradition (2022 – present). From left to right: PhD students Andrei-Mihai Vasilescu, Andra-Elena Coșoreanu-Șulea, Adina-Gabriela (Puiu) Vasilescu, Cătălin-Constantin Bica, and Professor Dr. Ștefan-Eugen Szedlacsek.

Building on these achievements, the Department advanced toward therapeutic bioconjugates by developing the concept of Affibody-Drug Conjugates (AfDCs). A recombinant anti-B7-H3 affibody fused with the cytotoxic peptide Magainin-2 was successfully expressed, purified, and tested in leukemic cell models, showing selective anti-AML activity and minimal off-target toxicity [30]. This approach represents a new therapeutic concept that combines recombinant fusion-based bioconjugate design with targeted cytotoxicity. In parallel, the Department explored nucleic acid-based therapeutics through the design of synthetic DNA minicircles aimed at inhibiting STAT3 activation in ovarian cancer cells. The resulting anti-STAT3 minicircle DNA effectively downregulated key oncogenic pathways, demonstrating a strong commitment to developing innovative molecular tools for cancer intervention [30, 31].

Collectively, these accomplishments positioned the Department as an emerging contributor to translational enzymology, where biochemical mechanisms intersect with biomedical innovation. They define a new paradigm in enzymology, one that connects the molecular understanding of enzymes with the development of clinical-grade translational tools, capable of transforming fundamental discoveries into tangible benefits for human health.

Acknowledgements: The authors express their gratitude to the former and current members of the Department of Enzymology whose dedication and research efforts over the years have shaped the scientific legacy described in this paper.

Authors' contributions: O.C.D. holds a background in economics with a master's degree in Management. Since 2015, she has coordinated the administrative and project management activities of the Department of Enzymology, providing essential logistical and organizational support. In this manuscript, she collected, organized, and edited the historical and bibliographic data extracted from the Department's electronic archive. S.-E.S. provided conceptual input, verified the scientific accuracy of the content, reviewed the manuscript, and refined the English language. Both authors discussed the structure of the article and approved its final version.

REFERENCES

1. SZEDLACSEK S.E, WASOWICZ E., HULEA S.A., NISHIDA H.I., KUMMEROW F.A., NISHIDA T., *Esterification of oxysterols by human plasma lecithin-cholesterol acyltransferase.,* J Biol Chem., 1995, 270, (20), 11812–11819.
2. SZEDLACSEK S.E., OSTAFE V., MOGOȘ S., HULEA S.A., *A kinetic method for the simultaneous determination of isoenzyme activities in mixture.*, Biochem Int., 1986, 12, (2), 279–289.
3. SZEDLACSEK S.E., *A re-evaluation of the kinetic equations for hyperbolic tight-binding inhibition.*, Biochem J., 1988, 254, 311–312.
4. SZEDLACSEK S.E., CARDENAS M.L., CORNISH-BOWDEN A., *Response coefficients of interconvertible enzyme cascades towards effectors that act on one or both modifier enzyme.s*, Eur J Biochem., 1992, 204, (2), 807–813.

5. SZEDLACSEK S.E., DUGGLEBY R.G., VLAD M.O., *Enzyme catalysis as a chain reaction.*, Biochem J., 1991, 279, 855–861.
6. VARÓN R., HAVSTEEN B.H., MOLINA-ALARCÓN M., SZEDLACSEK S.E., GARCÍA-MORENO M., GARCÍA-CÁNOVAS F. *Kinetic analysis of reversible closed bicyclic enzyme cascades covering the whole course of the reaction*., Int J Biochem., 1994, 26, (6), 787–797.
7. SZEDLACSEK S.E., DUGGLEBY R.G., *Kinetics of slow and tight-binding inhibitors.*, Methods in Enzymology., 1995, 249, 144–180.
8. BAICI A., SZEDLACSEK S.E., FRÜH H., MICHEL B.A., *pH-dependent hysteretic behaviour of human myeloblastin (leucocyte proteinase 3)*, Biochem J., 1996, 317, 901–905.
9. SZEDLACSEK S.E., ARICESCU A.R., HAVSTEEN B.H., *Time-dependent control of metabolic systems by external effectors.*, J Theor Biol., 1996, 182, (3), 341–350.
10. ARICESCU A.R., FULGA T.A., CIŞMAŞIU V., GOODY R.S., SZEDLACSEK S.E., *Intramolecular interactions in protein tyrosine phosphatase RPTPμ: kinetic evidence.*, Biochem Biophys Res Commun., 2001, 280, (1), 319–327.
11. BĂLAŞU M.C., SPIRIDON L.N., MIRON S., CRAESCU C.T., SCHEIDIG A.J., PETRECU A.-J., SZEDLACSEK S.E. *Interface analysis of the complex between ERK2 and PTP-SL*., PLoS One., 2009, 4, (2), e5432.
12. SZEDLACSEK S.E., ARICESCU A.R., FULGA T.A., RENAULT L., SCHEIDIG A.J., *Crystal structure of PTP-SL/PTPBR7 catalytic domain: implications for MAP kinase regulation.,* J Mol Biol., 2001, 311, (3), 557–568.
13. VLAD M.O., ROSS J., SZEDLACSEK S.E., *Fisher's theorems for multivariable, time- and state-dependent systems.*, Proc Natl Acad Sci U S A., 2005, 102, (42), 15061–15066.
14. DURSINA B., REENTS R., DELON C., WU Y., KULHARIA M., THUTEWOHL M., VELIGODSKY A., KALININ A., EVSTIFEEV V., CIOBANU D., SZEDLACSEK S.E., WALDMANN H., GOODY R.S., ALEXANDROV K., *Identification and specificity profiling of protein prenyltransferase inhibitors using new fluorescent phosphoisoprenoids.,* J Am Chem Soc., 2006, 128, (6), 2822–2835.
15. KÖHN M., TURNBULL A., DÜRSINA B., ELLMERICH S., KAUFMANN D., JACKSON D., ALEXANDROV K., SZEDLACSEK S.E., MANN M., SUPERTI-FURGA G., WALDMANN H., SCHELHAAS M., *A microarray strategy for mapping the substrate specificity of protein tyrosine phosphatases.*, Angew Chem Int Ed., 2007, 46, (39), 7700–7703.
16. PASCARU M., TĂNASE C., VACARU A.M., BOETI P., NEAGU E., POPESCU I., SZEDLACSEK S.E. *Analysis of molecular determinants of PRL-3.,* J Cell Mol Med., 2009, 13, (8A), 3141–3150.
17. VALERIU B.CISMASIU, STEFAN A.DENES, HELMUT REILANDER, HARTMUT MICHEL, STEFAN E.SZEDLACSEK., *The MAM (meprin/A5-protein/PTPmu) domain is a homophilic binding site promoting the lateral dimerization of receptor-like protein-tyrosine phosphatase mu*, J.Biol.Chem, 26922-–26931, 2004.
18. TABERNERO L., ARICESCU A.R., JONES E.Y., SZEDLACSEK S.E., *Protein tyrosine phosphatases: structure-function relationships*, The FEBS Journal, 2008, 275(4): 867–882.
19. PETRĂREANU G., PASCARU M., BĂLAŞU M., SZEDLACSEK S.E. *Preliminary X-ray crystallographic analysis of the D-xylulose-5-phosphate phosphoketolase from Lactococcus lactis.,* Acta Crystallogr Sect F Struct Biol Cryst Commun., 2010, 66, (8), 805–808.
20. MENTEL M., BADEA R.A., NECULA-PETRĂREANU G., MALLIKARJUNA S.T., IONESCU A.E., SZEDLACSEK S.E. *Expression, purification, and kinetic analysis of PTP domains*., Methods Mol Biol., 2016, 1447, 39–66.
21. MENTEL M., IONESCU A.E., PUSCALĂU-GÎRTU I., HELM M.S., BADEA R.A., RIZZOLI S.O., SZEDLACSEK S.E., *WDR1 is a novel EYA3 substrate and its dephosphorylation induces modifications of the cellular actin cytoskeleton*., Sci Rep., 2018, 8, 2910.
22. IONESCU A.E., MENTEL M., MUNTEANU C.V.A., SIMA L.E., MARTIN E.C., NECULA-PETRĂREANU G., SZEDLACSEK S.E. *Analysis of EYA3 phosphorylation by Src kinase identifies residues involved in cell proliferation*., Int J Mol Sci., 2019, 20, (24), 6307.

23. PETRĂREANU G., PASCARU M., BĂLAȘU M., SZEDLACSEK S.E. *Phosphoketolases from Lactococcus lactis, Leuconostoc mesenteroides and Pseudomonas aeruginosa: dissimilar sequences, similar substrates but distinct enzymatic characteristics*., Appl Microbiol Biotechnol., 2014, 98, (18), 7855–7867.
24. SCHEIDIG A.J., HORVATH D., SZEDLACSEK S.E., *Crystal structure of a xylulose-5-phosphate phosphoketolase: insights into the substrate specificity for xylulose-5-phosphate*., J Struct Biol., 2019, 207, 85–102.
25. MANOLACHE A., SELESCU T., MAIER L.G., MENTEL M., IONESCU A.E., NEACȘU C., BABEȘ A., SZEDLACSEK S.E., *Regulation of TRPM8 channel activity by Src-mediated tyrosine phosphorylation.*, J Cell Physiol., 2019, 234, 1–12.
26. SZEDLACSEK H.Ș., BAJUSZ D., BADEA R.A., POP A., BICĂ C.C., NECULA-PETRĂREANU G., MUNTEANU C.V.A., KESERŰ G.M., SZEDLACSEK S.E., *Designed peptide inhibitors of STEP phosphatase – GluA2 AMPA receptor interaction enhance the cognitive performance in rats*., J Med Chem., 2021, 64, (24), 18623–18637.
27. SZEDLACSEK Ș.E., BADEA R.A., SZEDLACSEK H.Ș., HRITCU L., *Interference peptides as inhibitors of interactions related to AMPAR endocytosis.*, U.S. Patent No. 11834518 B2., 2023.
28. VÁRADI B., BREZOVCSIK K., GARDA Z., MADARASI E., SZEDLACSEK H., BADEA R.A., VASILESCU A.M., PUIU A.G., IONESCU A.E., SIMA L.E., MUNTEANU C.V.A., CĂLĂRAȘ S., VÁGNER A., SZIKRA D., TOAN N.M., NAGY T., SZŰCS Z., SZEDLACSEK S., NAGY G., TIRCSÓ G., *Synthesis and characterization of a novel [52Mn]Mn-labelled affibody-based radiotracer for HER2+ targeting*., Inorg Chem Front., 2023, 10, (9), 1234–1245.
29. VÁRADI B., SAJTOS G.Z., BREZOVCSIK K., SZŰCS Z., SZEDLACSEK S., NAGY G., TIRCSÓ G., *Mn(II)-based diagnostic agents: from basic research to targeted diagnostic procedure.*, Scientia et Securitas., 2024, 5, (2), 253–266.
30. VASILESCU A.-M., VASILESCU A.-G., SIMA L.E., MUNTEANU C.V.A., BARAN N., SZEDLACSEK S.E., *A novel cytotoxic anti-B7-H3 affibody with therapeutic potential in acute myeloid leukemia.*, Front Pharmacol., 2025, 16, 1–13.
31. VASILESCU A.-G., VASILESCU A.-M., SIMA L.E., BARAN N., SZEDLACSEK S.E., *Breaking the cancer code: a novel DNA minicircle to disable STAT3 in ovarian cancer cells SKOV3.*, Front Pharmacol., 2025, 16, 1–15.