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Expression of interest

of the **Institute of Biochemistry (IBAR), Bucharest, Romania**, through the **Department of Enzymology (DoE)**, to join a Consortium on the HORIZON EUROPE call:

HORIZON-CL6-2024-BIODIV-01-1
Invasive alien species
(https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/opportunities/topic-details/horizon-cl6-2024-biodiv-01-1)

Organization details:
Country: **ROMANIA**
Name of the organization: **Institute of Biochemistry of the Romanian Academy (IBAR)**, **Bucharest, Romania**/ **Department of Enzymology (DoE)**

Contact person short description and contact details: **Professor STEFAN EUGEN SZEDLACSEK, PhD; Head of Department**

1. **Short description of the Institute of Biochemistry (IBAR)**

IBAR - the institute was established in 1990 in order to develop and promote advanced research in biochemistry and molecular biology in Romania. It is funded by the Romanian Academy and the Romanian Government, through competitive grants awarded by the Executive Unit for Financing Higher Education, Research, Development and Innovation. For 32 years, it has been promoting **Protein Science** in Romania, by carrying out projects related to protein function, synthesis and trafficking with practical impact in biomedicine, nano- and biotechnologies. IBAR hosts a state-of-the-art research center in biomedical proteomics, operated by an interdisciplinary team of young researchers with studies in biochemistry, chemistry, biophysics, biology, and pharmacology. As part of its infrastructure, the institute has 3 proteomics labs, 3 cell culture labs, one microscopy lab, one virology lab and one bioinformatics center and it is constituted of 5 departments: Enzymology, Molecular Cell Biology, Ligand-Receptor Interactions, Viral Glycoproteins and Bioinformatics & Structural Biochemistry (https://www.biochim.ro/).

The institute has participated and is involved in numerous international and national collaborations, of which a few projects of note are:

- EEA Grants (2014-2021): Next Generation Viral Hepatitis B and C vaccine development in plants and algae using advanced biotechnological tools (SmartVac), project ID: **EEA-RO-NO-2018-0078** (https://www.smartvac.ro/), in collaboration with The Norwegian Institute of Bioeconomy Research (NIBIO), Norway, The Center for Infection and Immunity of Lille (CIIL), France and The "Cantacuzino" National Institute for Medico- Military Research and Development (INCDMM "Cantacuzino"), Romania
- Role of TG2 in cancer tumor microenvironment for guiding metastasis prevention therapeutic approaches (2020-2022) (TG2TARGET), project ID: **PN-III-P1-1.1-TE-2019-0670** (http://www.biochim.ro/grant-29-tg2target/)
- High-throughput screening platform for small-molecules with anti-inflammatory potential (HTS-IL-1β) (2020-2022), project ID: **337PED/2020 PN-III-P2-2.1-PED-2019-3297** (https://marichiritoiu4.wixsite.com/ped337), in collaboration with National Institute of Pathology "Victor Babeş" (IVB), Romania

Possible main contributions of the **Institute of Biochemistry of the Romanian Academy (IBAR**), **Bucharest, Romania**/ Department of Enzymology (DoE) **to the** HORIZON-CL6-2024-BIODIV-01-1 **call:**

- WORK PACKAGE LEADER **X**

We are confident that we can bring a significant contribution to the topic **"Invasive alien species"** through the extensive expertise that our department accumulated over the years about protein characterization and design. Although our main efforts focused on enzymes present in the human body: elucidating protein tyrosine phosphatases [Ionescu, A.E. et al. "Analysis of EYA3 Phosphorylation by Src Kinase Identifies Residues Involved in Cell Proliferation"; Int. J. Mol. Sci. 2019, 20, 6307. https://doi.org/10.3390/ijms20246307] and designing peptide inhibitors for STEP phosphatase found in the brain [Szedlacsek, H.S. et al. "Designed peptide inhibitors of STEP phosphatase-GLUA2 AMPA receptor interaction enhance the cognitive performance in rats". J Med Chem. 2022 jan 13;65(1):217-233. doi: 10.1021/acs.jmedchem.1c01303], our analysis also extended to microorganisms [Petrareanu G et al. "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. https://doi.org/10.1007/s00253-014-5723-6]. Thus, we are confident that our expertise and technical capabilities could help build more defined profiles of invasive species present in both terrestrial and aquatic ecosystems and facilitate adopting an adequate strategy for biodiversity conservation.

We suggest four potential contributions to this topic:

1. Performing of a full proteomic analysis of given alien species, using our expertise and nanoLC-MS/MS infrastructure;
2. Identification of critical and specific molecules of alien species (e.g. specific enzymes which are essential for the survival of studied, alien species);
3. Cloning the coding genes, expressing, purifying and biochemically characterizing corresponding proteins from invasive alien species;

4. Designing inhibitors against identified molecules, critical to population control of invasive alien species (in collaboration with specialists in structure-based, computer-aided design).

Specific relevant expertise of Department of Enzymology (DoE)

The Department of Enzymology's main activity is conducting both fundamental and applicative research. Its central research topic is the study of structure-function relationships in signaling enzymes. The aim is to contribute to the understanding of how their structural features are correlated with specific signaling functions. DoE has 32 years of experience and is currently involved in the production, isolation and purification of recombinant proteins, expressed in both prokaryotic and eukaryotic systems. The activity of the research group is carried out with tools of molecular biology (recombinant DNA, site-directed mutagenesis, (RT)-PCR, Western blot, immunoprecipitation, etc.), spectroscopic analysis (UV-VIS and fluorescent spectrophotometry), cell biology, protein crystallization and enzyme kinetics. The DoE team demonstrates expertise and the highest standards of research practices in areas of biochemistry, molecular biology and medical biotechnology. In recent years, they developed small protein molecular vehicles called affibodies, targeting human epidermal growth factor receptor 2 (HER2) in breast cancer. The anti-HER2 affibody labeled with ^{52}Mn has been used in PET imaging revealing HER2+ tumours in mouse xenograft models (https://doi.org/10.1039/D3QI00356F). An important step towards clinical translation made by the Enzymology team was the design and synthesis of potent neuropeptides that can inhibit STEP function in dementia rat models and enhance cognition. The developed peptides are patent pending in the USA (DOI 10.1021/acs.jmedchem.1c01303). Furthermore, our group has significant expertise in cell signaling research with a focus on protein tyrosine phosphatases (PTPs). In 2018-2019, we demonstrated the role of EYA3 (a PTP) in actin cytoskeleton signaling processes and therefore, its implication in cancer progression and metastasis. This work shows for the first time the specific sites where EYA3 is phosphorylated by Src kinase. Also, it reveals WDR1 (an important cytoskeleton regulator) as a novel cytoplasmic substrate of EYA3, which is at the same site phosphorylated by Src and dephosphorylated by EYA3 (DOI 10.3390/ijms20246307; DOI 10.1038/s41598-018-21155-w). Finally, as an example of our long-term dedication to cancer research, our team leader, Professor Szedlacsek, had a crucial role in elucidating the nucleocytoplasmic localization and transport mechanisms of PRL-3 (an important PTP involved in cancer metastasis), as well as mechanisms for its catalytic regulation (DOI: 10.1111/j.1582-4934.2008.00591.x).

Department Head - Professor Stefan E. Szedlacsek has been involved and is currently engaged together with the DoE in numerous international and national projects and collaborations with high impact and current relevance in biology research and the biomedical sciences and industry. Some recent examples include:

- Bilateral agreement (2022-2024): Radiolabelling of affibody for tumor diagnostic and theranostic application in the nuclear medicine; project number: 2886/15.09.2021 (https://www.biochim.ro/grant-37-bilateral-agreement-no288615092021-romanian-

academy-hungarian-academy-of-sciences/) between IBAR, Institute for Nuclear Research from the Hungarian Academy of Sciences (ATOMKI) and University of Debrecen

- EEA Grant (2021-2023): Next generation of drug targets for schizophrenia (NEXTDRUG); project ID: EEA-RO-NO-2018-0535 (https://eeagrants.org/archive/2014-2021/projects/RO-RESEARCH-0034) between IBAR, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway and The "Cantacuzino" National Institute for Medico- Military Research and Development (INCDMM "Cantacuzino"), Romania
- An important collaboration (2015-2018) with Professor Silvio Rizzoli from the Department for Neuro- and Sensory Physiology, University Medical Center Göttingen and the Center for Nanoscale Microscopy and Molecular Physiology of the Brain, Germany, in which they showed WDR1, an important cytoskeleton regulator, as a novel substrate of PTPase EYA3, with significant implications in diverse malignancies (DOI 10.1038/s41598-018-21155-w)

As part of IBAR, our department has access to modern infrastructure, highly valuable in proteomics and cell biology: ÄKTA PRIME™ (Cytiva) Chromatographic System – for protein purification; BIACORE™ 3000 (Cytiva) – Surface plasmon resonance – for biocompound interaction studies; LTQ Orbitrap Velos Pro™ II nanoLC-MS system (Thermo Scientific) – mass spectrometric analysis; Nanodrop™ 2000 Spectrophotometer (Thermo Scientific) – for DNA and protein quantification; FLUOStar™ Omega Microplate reader (BMG Labtech) – for spectrophotometric and spectrofluorimetric measurements; FACSVerse™ Flow cytometer analyser (BD Biosciences); Confocal LSM 710 Microscope (ZEISS); Protein gel electrophoresis systems (Bio-Rad) – for protein separation in polyacrylamide gels; Protein wet transfer system (Bio-Rad) – for Western Blotting; Class II Biological Safety Cabinets (Heraeus) and CO2 incubator (Revco) – for cell culture experiments;; BioRad DNA Engine Dyad Peltier Thermal Cycler - Real Time qRT PCR; Chemidock – for visualising WB results.

DoE - (https://www.biochim.ro/group-enzymology/)

IBAR Infrastructure - (https://eeris.eu/ERIO-2000-000X-0177)

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