CA21111 – One Health drugs against parasitic vector borne diseases in Europe and beyond (OneHealth*drugs*)

Short-Term Scientific Mission Grant
- EXTENDED RESEARCH PROPOSAL[1] -

Action number: 21111

Applicant name: Aleksandar Cvetkovski

Specify on which deliverable the Applicant will be working on during the prospected STSM:

Working Group 1:	**Compound libraries coordination and integration of compound design**
Working Group 2	**Integration of early phase studies and low environmental impact actions**
Working Group 3	**Coordination of in vitro-to-in vivo translation of OneHealth leads and candida**
Working Group 4	**Integration of R&D process-environmental studies and translation in informed whitepaper**

Details of the STSM

Title: Modelling of binding affinity of siderophores with redox **Fe^{3+}/Fe^{2+}** system as a potential new class of anti-parasitic drugs

Start and end date: 19/08/2024 to 31/08/2024

Nr. of days: 13 (maximum 180 days)

Requested budget

a) 500 Euro for travel
b) 1900 Euro for stay and meals (to justify)
This amounts to 51 euros per day, which is in the lower end for possible accommodation and meals in Estonia. Tartu is a university city that has a long deficit of accommodation that sees prices for rooms to be very high. Meals and living costs are also high, comparable to Northern Europe.
c) 1163 Euro total budget (=a+b)

Please refer to the table below:

	STSM GRANT			
	mission duration			
Hosting Lab Country	**days <30**	**30< days <60**	**60< days <120**	**120< days <180**
Member and Cooperating Country	1.200 €	2.400 €	3.400 €	4.000 €
African and Caribbean Country	2.000 €	3.000 €	4.000 €	4.000 €

Home institution:

[1] This form is part of the application for a grant to visit a host organisation located in a different country than the country of affiliation. It might contain information already present in the Application form, please repeat them. It is submitted to the COST Action MC via e-COST. The Grant Awarding Coordinator coordinates the evaluation on behalf of the Action MC and informs the Grant Holder of the result of the evaluation for issuing the Grant Letter.

Name of the applicant: Prof (Assoc). Dr. Aleksandar Cvetkovski, PhD
Affiliation: University "Goce Delcev" Stip
Faculty of medical science
Krste Misirkov b.b.
P. fax. 201, 2000 Stip
Country: Republic of N. Macedonia

YRI: yes**/no** (YRI = Young Researcher and Innovator, 40 years old maximum, as indicated in the COST Action rules)
Please provide a letter/email from the head of the home institute/University laboratory attesting for the full salary of the STSM applicant during the training abroad and for agreeing with the project dates and focus.

Host institution:
Name: Prof. (Assoc.) Dr. Alfonso T. García-Sosa, PhD
Affiliation: Institute of Chemistry, University of Tartu
Country: Ravila 14a 50411Tartu
Please provide a letter/email of the host that confirms the intent to host the STSM applicant.

Summary

A lack or ineffective treatments and vaccines for some diseases like malaria and cancer concerns and challenges public health issues nowadays to provide effective and efficiency therapy regimens toward class new classes of drugs with novel mechanisms of action [1]. In 2020, there were an estimated 241 million cases of malaria worldwide, and the estimated number of deaths stood at 627,000 [2]. Because the fight against this disease is focused on controlling the *Anopheles* mosquito that transmits the parasite [3] and on vaccination, many of these works have not advanced. Nevertheless, the recent approaches in chemoprevention [4] make necessary the development of new alternatives in malaria treatment. Siderophores are low-molecular-weight secondary metabolites that function as iron chelators offer a viable alternative to face these clinical problems. Under iron-deficiency conditions, they are produced by a wide variety of microbes, allowing them to increase their iron uptake. The primary function of these compounds is the environmental iron scavenging and its transport into the cytosol. Depending on their functional group, siderophores are classified into hydroxamate, catecholate, phenolate, carboxylate, and mixed types. They have achieved great importance in recent years due to their medical applications as antimicrobial, antimalarial, or anticancer drugs, vaccines, and drug-delivery agents.

Goals of the STSM

To study the molecular and structural classes of siderophores that form complexes with Fe^{2+} and thus, may affect inhibition of metabolic processes in parasites.

Siderophores are low-weight, high-affinity iron chelating molecules, produced as secondary metabolites. They detect sufficient levels of iron supply for infection by the *Plasmodium falciparum* parasite and respond as inhibitor to the essential metabolic processes of depriving the parasite of iron as a control measure for malaria. [5, 6] Iron acquisition by bacterial siderophores as virulence factor requires to target the potential inhibition sites (e.g., siderophore biosynthesis, outer membrane receptors, iron release enzymes, and signal receptors). [7] The iron acquisition process starts recognizing the Fe^{3+} complexes by cell surface receptors and ends in reduction of Fe^{3+} into Fe^{2+} form, which then enters the cytoplasmic metabolic path, i.e., "plasmocidin" activity of endocellular parasites, in particular those contained in red blood cells, and is not reversed by iron. Despite many advances, the exact role of Fe acquisition systems *in vivo* and their effects in pathogenic virulence remain to be determined. [8].

Working Plan

To comprehensively address the steps and objectives outlined, a detailed working plan has been devised:

1. Database Exploration and Selection:
- Identify available databases with crystal & molecular structures of siderophores. Some are available in the Protein DataBank, ChEMBL, PubChem, DrugBank, MOAD, BindingDB, as well as the CCDC collection of small molecules.
- Evaluate the quality, comprehensiveness, and relevance of the entries retrieved from each database.
- Select the most appropriate structures for further analysis and modelling.

2. System Construction:
- Construct protein-metal-ligand systems with ligand functional group modifications to understand the intra- and intermolecular interactions and behaviours.
- Design metal-ligand systems, considering these unique properties and characteristics of siderophores.

3. Ligand and Protein Modelling:
- Utilize molecular modelling techniques, such as:
  - Docking to predict the preferred orientation of the ligand when bound to a protein to form a stable complex.
  - Molecular dynamics to simulate the behaviour of molecules.
- Estimate the free energy of binding using specialized methods to determine the binding affinity between ligands and proteins. [9]

4. Results Interpretation:
- Analyse the obtained data, identifying patterns, including statistics, and drawing conclusions about siderophore behaviour and interactions.

5. Method Development :
- Use the affinity/binding constants to create relationships that correlate thermodynamic data with crystallographic data for siderophore-iron complexes. Building Quantitative Structure-Activity Relationships of the ligands.
- Collaborate with WG1 to source and update the compound library.
- Partner with WG3 to either simulate or conduct experiments for in vitro/in vivo testing.

6. Mechanism Analysis:
- Delve deep into understanding the action mechanism (MoA) of siderophores. Reject hypothesis about possible MoAs that do not explain activity.
- Rationalize how the activity of siderophores can be harnessed for therapeutic purposes.

7. Drug Development Ideas:
- Propose concepts for advancing siderophores as antibiotics targeting vector-mediated diseases making use of their inhibition of Fe systems in organisms. There exists available data on the use of siderophores for iron overload disease in humans.
- Explore the potential pathways for drug development, considering the unique properties of siderophores.

8. Knowledge Sharing:
- Draft research articles detailing the findings.
- Prepare presentations to share insights at scientific conferences and gatherings.
- Establish communication channels with working group 5.
- Disseminate and communicate findings through events and on social media.
- Ensure that the knowledge reaches relevant stakeholders and contributes to the broader scientific community.

Expected outcome and contribution to the Action MoU objectives and deliverables

To provide data sets of affinity/binding constants that offer development of algorithms for correlation thermodynamic to crystallography data of siderophore-iron complexes that acts as antiparasitic drugs and assessment of their further in *vitro/in vivo* testing for efficacy and safety (collaboration with WG1 on collecting the library for compounds, and with WG3 on simulation or perform experiments of in vitro/in vivo testing)

To rationalize the activity and mechanism of action of siderophores.

To provide drug design ideas for further development of siderophores as antibiotics for vector-mediated diseases.

Dissemination of knowledge and results (collaboration with WGs 4&5) toward the further collaboration with partners from pharma/chem industry for synthesis of Active Pharmaceutical Ingredients (APIs), possible issuing patent (Intellectual Property Right Protection), publication research paper and presentation on the conferences.

References

1. Khasheii B, Mahmoodi P, Mohammadzadeh A (2021) Siderophores: importance in bacterial pathogenesis and applications in medicine and industry. Microbiol Res 250:126790. https://doi.org/10.1016/j.micres.2021.126790
2. WHO (2022) World malaria report. https://www.who.int/publications/i/item/9789240064898. Accessed 11 May 2023
3. Sougoufara S, Ottih EC, Tripet F (2020) The need for new vector control approaches targeting outdoor biting anopheline malaria vector communities. Parasit Vectors 13(1):295. https://doi.org/10.1186/S13071-020-04170-7
4. Greenwood B, Cairns M, Chaponda M, Chico RM, Dicko A, Ouedraogo JB, Phiri KS, ter Kuile FO, Chandramohan D (2021) Combining malaria vaccination with chemoprevention: a promising new approach to malaria control. Malar J 20(1):1–7. https://doi.org/10.1186/S12936-021-03888-8
5. Maya-Maldonado K, Cardoso-Jaime V, González-Olvera G, Osorio B, Recio-Tótoro B, Manrique-Saide P, et al. (2021) Mosquito metallomics reveal copper and iron as critical factors for *Plasmodium* infection. PLoS Negl Trop Dis 15(6): e0009509. https://doi.org/10.1371/journal.pntd.0009509
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8. Helen L Collins, The role of iron in infections with intracellular bacteria, **Immunology Letters** 2003Vol. 85, 2, 193-195 https://doi.org/10.1016/S0165-2478(02)00229-8
9. Borges, A.; Simões, M.; Todorović, T.R.; Filipović, N.R.; García-Sosa, A.T. Cobalt Complex with Thiazole-Based Ligand as New *Pseudomonas aeruginosa* Quorum Quencher, Biofilm Inhibitor and Virulence Attenuator. *Molecules* **2018**, *23*, 1385. https://doi.org/10.3390/molecules23061385 https://doi.org/10.3390/molecules23061385

1. Home lab description

The Faculty of Medical Sciences represents the largest faculty at the Goce Delchev University in Stip, North Macedonia. Located on Campus 3 within the university framework, the Faculty of Medical Sciences offers opportunities to pursue three academic studies spanning integrated first and second cycles, Master Level (general medicine, dental medicine, pharmacy) and six professional studies in the first cycle (nursing, midwifery, dental technicians, medical laboratory technicians, physiotherapists, and optometrists). In addition to first-cycle studies, the Faculty of Medical Sciences also provides opportunities for second and third-cycle (PhD) studies. The Faculty of Medical Sciences prioritizes inclusivity, diversity, collaboration, education, innovation, and social well-being. It aims to address the scientific challenges of the 21st century with a student-centred approach. Known for its discoveries, creativity, innovation, and knowledge dissemination for medical and economic prosperity, the faculty promotes open-mindedness, understanding, compassion, and inclusivity through various activities encompassing knowledge, art, culture, and sports.

University central core lab facilities' offer access for biomedical research in drug discovery and development (crystallographic lab, imaging techniques lab, analytical services lab, radiopharmaceutical lab), preclinical research, eco-toxicology and environmental protection research in public healthcare, and clinical trials in collaboration with University Clinical Centre in Satip, and partner hospitals

2. Hosting Lab description

Our lab performs research in computational chemistry, molecular modeling, data science, XAI/ML, data curation, model generation and validation, compound design and analysis, bioinformatics, supervision of staff and doctoral students.

Strong part of 60 WoS publications in prestigious journals in the field, 89% of which the PI is first and/or corresponding author. 30 are from projects led by the PI. These are results and developments in:

Design of inhibitors for HIV reverse transcriptase, integrase, and protease, Leishmania (several targets), avian influenza H5N1, antibacterial film inhibitors, antioxidative and neuroprotective agents (aging related diseases), myosin inhibitors, cancer-related programmed death-ligand 1 (PD-L1), CDK2, and PARP.

Design of cardioprotective compounds and for heart failure treatment (WO and EP patents 2010).

Mechanisms of action investigation in human, pathogen, and plant systems.

Design of siRNA cargo delivering cell-penetrating as well as antimicrobial peptides.

Water molecule large crystal structure analysis and their use in free energy perturbations and drug design.

Improvement of protein-ligand binding scoring functions by better use of scoring function terms and normalization.

Machine learning and data science in chemistry and drug design on different organs/tissues, as well as toxicity prediction through

DNNs, RF, and Graph-convolutional NN (CNN) on androgen receptor binding, made publicly available and reusable on github

Drug discovery and development research requires better methods for predicting active compounds. Developed and applied improved methodology and approaches such as antitargets, consensus, and reranking in virtual screening and molecular docking, that have led to the design of compounds for activity against Leishmania.

Better use is needed of the vast amounts of biological and chemical data that are becoming increasingly available for chemical compounds. Drug/nondrug and disease organ and tissue classification methods have been developed, and modification of other mathematical approaches in chemistry and pharmacology (naive Bayesians, logistic regression, PCA, probability distribution ratios) that allow the better profiling, classification for better targeting and use of chemicals and libraries. Developed new ranking methods for ligand affinity.

Toxicology model development and curation. Led the Human Health part of the ChemInDisco work on the largest survey, analysis, and making publicly accessible of all publications using QSAR, QSPR, and QS*R predictions and modeling and curated and uploaded onto the qsardb.org. This resulted in the recent database and public establishment of best practices for QSAR model reporting for physical and chemical properties, ecotoxicity, environmental fate, human health, and toxicokinetics endpoints.

Leading computational biochemistry part in projects EU FP7 Chemomentum (publications, OpenMolGrid development), and EU FP7 Cardioworkbench (cardiovascular drug design, 2 patents)

Reviewer for over 37 journals in the field

Editorial Board Member of 3 journals in the field

Over 1,820 citations to my work and over 100 citations in books

MC member and leading projects in the EU COST Actions MuTaLig, Stratagem, CardioRNA, Cardioprotection, and Anti-parasitic chemotherapy (PI is the member for Estonia in all of these) for research, training young scientists, synthesis of compounds, testing of chemical compounds and modeling methods developed, collaboration and outreach with companies.
Created, managed, and led a research team of up to five people.
Proposed research themes, provided and managed resources, supervised research projects, presentations, and publications.
Milan Sencanski, Senior Researcher from the VINCA Institute, Belgrade carried out a research stay in 2018 under the PI's supervision and funded by the Archimedes Foundation.
José Peña-Guerrero, PhD student from the University of Navarra that the PI supervised in two research stays at the University of Tartu during 2018 and 2019 (six months). Microbiologist by training, he gained expertise in computational biology and computational chemistry, conducting protein search and identification, homology modelling, protein structure evaluation, virtual screening, molecular dynamics, protein-protein docking, compound library testing, analog compound identification and testing.
Alexis Galán Rodríguez, PhD student from the University of La Laguna, Canarias, Spain that carried out a research project on docking, molecular dynamics, and free energy methods I supervised at the University of Tartu.
Christina Koumpoura, PhD student from University of Toulouse, France, 2022, synthetizing Lawson derivatives by mechanochemistry and calculating their interactions with their target.
Filipe Estrada, Medicinal chemistry Master's student from the University of Lisbon the PI supervised at the University of Tartu, 2019, gained expertise in protein-ligand interaction fingerprints, docking, MD simulations, virtual screening.
Edgar Rosales, Pharmacy Master's student from UAEM, Mexico the PI co-supervises with a project to develop benzylpiperidines as therapeutic agents for neurodegenerative disease and neuropathic pain.

Infrastructure present:
Schrödinger Suite 2023, Gaussian, GaussView, Gromacs, Simca-P, MODDE, ChemOffice Ultra, Autodock, Gromos96, AMBER16, CODESSA, MOPAC, R, protein-protein interaction, molecular geometry optimization, docking, HTVS, property determination, QSAR building & validation, quantum mechanics. In-house developed software (Chemomentum, QsarDB). Server room with 370 CPU cores and several GPUs, application servers, 2 database & 2 storage servers with 66 TB of disk space. Torque resource manager & UNICORE grid middleware. 19 workstations.
Dedicated labs w/ central & autonomous AGA gas-delivery lines & regulators, central multiple-outlet vacuum system, rotary evaporator (Buchi) w/ dry vacuum-pump (VacuumBrand), benchtop 2-hand glovebox (PlasLabs), heating plates (IKA)w/ magnetic stirrer & digital contact-thermometer. Ice generator (Laser-Film), column & TLC, high-precision peristaltic pumps (Cole Parmer), multiple freq. UV source (Spectroline). UV/VIS spectrophotometer Spectrostar (BMG), supercritical CO2 extraction equip. (DIONEX), surface area & pore size (Quantachrome), pH-meter (Mettler-Toledo)

3. Hosting Lab selection motivation

The hosting lab has strong experience in protein-ligand interaction and drug design. The PI has over 60 publications in the field. Several projects are related to OneHealth principles, as well as collaborations with other members of the COST Action 21111 OneHealthdrugs. The PI has experience in supervising and coordinating junior and senior scientists in research projects in the field. The PI has also successful experience in hosting STSMs.

The applicant can also develop scientific collaborations during his stay at the University of Tartu.

Dissemination plans

At least one research paper and conference presentations according to CA21111 annual plan.

Presenting at science festivals

Recording a video presentation of the researchers and work performed

Use of social media to communicate material related to the STSM and COST Action 21111

Benefits of the STSM

To launch the platform for rational approach in drug design based on optimisation the lead compounds based on searching the toward the library/ datasets of affinity/binding constants calculated based on correlation structural and thermodynamic parameters.

To shortcut the drug development and focus on in *vivo/in vitro* testing the efficiency and safety of drug candidates and thus, contribute to better drug discovery for vector'mediated diseases.

Prospective evaluation of prospective ligands towards their ecological and toxicological effects.

To develop highly-qualified human resources by increasing the applicant's knowledge and skills in the computational chemistry of protein-ligand systems related to OneHealth.

Please read the following guidelines and delete them before submitting your Extended Research Proposal.

How to apply on the COST web platform and supporting documentation

STSM – supporting documentation

- STSM grant Application (based on e-COST template*)

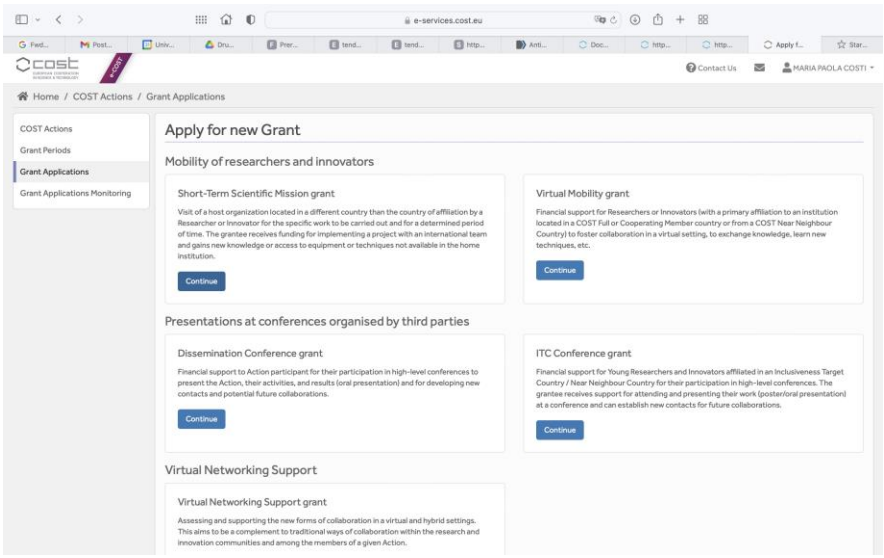
  - enter your personal e-cost page
  - Go to Grant application
  - Select the grant type
  - Select the Action CA21111
  - Proceed by filing the grids

- Confirmation of the host on the agreement from the host institution in receiving the applicant

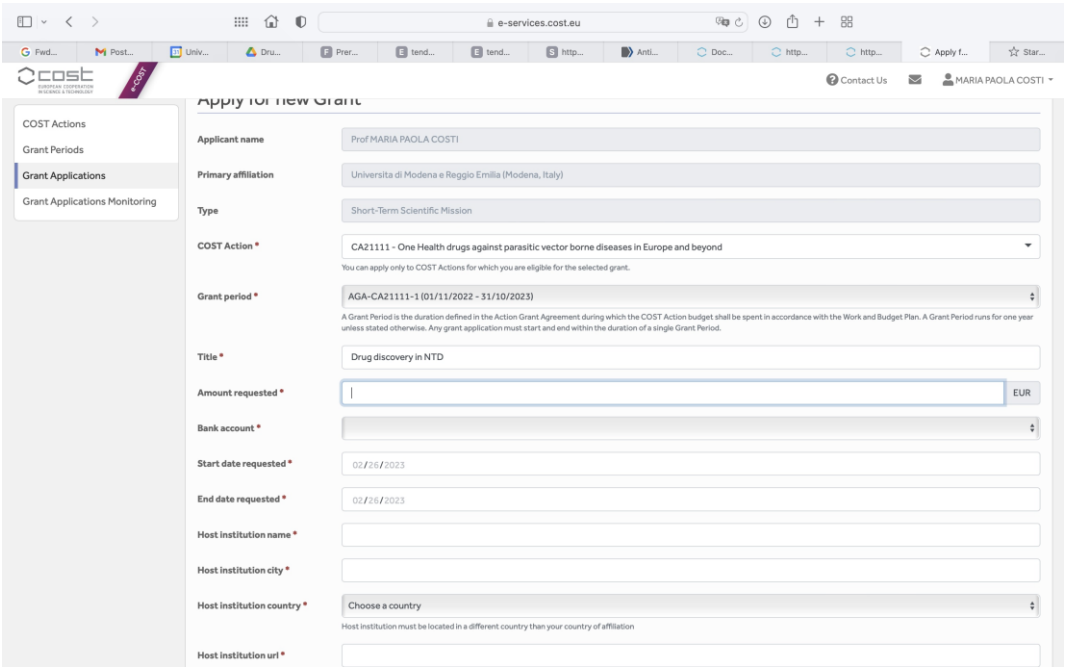
- Other documents required by the Action

***COST Action web platform to access the Grant application**

Step 1



Step 2



Read Carefully the Grant awarding User guide. This is enclosed, but it can also be found in the cost website.