MARIE SKŁODOWSKA-CURIE POSTDOCTORAL FELLOWSHIPS 2024

EXPRESSION OF INTEREST FOR HOSTING MARIE CURIE FELLOWS

HOST INSTITUTION

ITQB NOVA

RESEARCH GROUP AND URL

Membrane Protein Crystallography Lab –

Sulfur Metabolism in Human Pathogens Group (https://www.itqb.unl.pt/researchers/jbrito)

SUPERVISOR (NAME AND E-MAIL)

José Artur Brito – jbrito@itqb.unl.pt

SHORT CV OF THE SUPERVISOR

Jose Artur Brito graduated in Biotechnological Engineering in 2001 and after a short training period at the University of Madison Wisconsin (USA) he joined the Macromolecular Crystallography Lab at ITQB NOVA. After his *Ph.D.* degree in 2011, Jose took a joint postdoctoral position with the Membrane Protein Crystallography and the Bacterial Cell Biology labs at ITQB NOVA and in 2018, he became co-Principal Investigator of the Membrane Protein Crystallography Laboratory.

Jose's work lies on the interface between Biochemistry and Biophysics, with a special emphasis on Structural Biology techniques. His Group is interested in exploring alternative metabolic pathways in pathogenic bacteria, namely, hydrogen sulfide synthesis and degradation, thiosulfate/tetrathionate interconversions, and RNA homeostasis, as promising drug targets for new antibiotics. Jose has been studying some of the proteins from these pathways that might present as potential novel drug targets, which can, I turn, be used to tackle antibiotic resistance.

5 SELECTED PUBLICATIONS

- Brito, J.A. et al., Biochemistry, vol. 48, no. 24, 2009 - https://pubs.acs.org/doi/10.1021/bi9003827
- Walsh, B. J., S. S. Costa, K. A. Edmonds, J. C. Trinidad, F. M. Issoglio, J. A. Brito, D. P. Giedroc, Antioxidants (Basel). 2022 Aug 19;11(8):1607 https://www.mdpi.com/2076-3921/11/8/1607
- Kurth, J. M. et al., J. Biol. Chem., vol. 291, no. 48, 2016 https://pubmed.ncbi.nlm.nih.gov/27694441/
- B. J. C. Walsh, J. A. Brito, and D. P. Giedroc, "4.17 - Hydrogen Sulfide Signaling and Enzymology," in Comprehensive Natural Products III, H.-W. (Ben) Liu and T. P. Begley, Eds. Oxford: Elsevier, 2020, pp. 430–473 https://www.sciencedirect.com/science/article/abs/pii/B9780124095472146992?via%3Dihub
- Y. Zhang, G. Gonzalez-Gutierrez, K. A. Legg et al., Nat Commun 13, 7586 (2022) https://www.nature.com/articles/s41467-022-35277-3

PROJECT TITLE AND SHORT DESCRIPTION

Functional and structural studies on a transmembrane ABC transporter specific for ergothioneine uptake in bacteria

Infectious disease remains an ongoing threat to human health, with an increasingly urgent need to develop new antimicrobial strategies that limit the impact of life-threatening bacterial pathogens. An improved mechanistic understanding of bacterial adaptation to host-imposed stressors is critical to the development of these new strategies.

Cell-abundant low molecular weight (LMW) thiols maintain the reducing environment of the cytoplasm of bacterial cells and the cytosol of eukaryotic cells and include the ubiquitous tripeptide glutathione. Bacteria unable to synthesize or import glutathione synthesize other thiols, including bacillithiol and mycothiol found in some Firmicutes and Actinomycetes, respectively. These abundant LMW thiols scavenge endogenous or exogenous reactive oxygen and nitrogen species (ROS, RNS), creating thiol disulfides that are subsequently enzymatically reduced, thus maintaining redox balance. Bacterial LMW thiols are widely known to play critical roles in oxidative, reductive, electrophile and transition metal stress responses in the infected host.

Ergothioneine (ET) is an unusual LMW thiol and 2-thio derivative of trimethyl-*L*-histidine that is receiving significant recent attention as a potent antioxidant and cytoprotectant of therapeutic value. In vertebrates, ET is obtained from the diet and becomes broadly bioavailable in tissues and cells via the action of the ubiquitously expressed ET transporter (ETT). ET is biosynthesized by select fungi and bacteria from histidine, notably *Mycobacterium tuberculosis* (*Mtb*), where it may function as a scavenger of ROS and is a known virulence determinant. However, the extent to which ET broadly functions in bacterial cells, in particular human pathogens unable to synthesize it, is unknown. The Lab of Prof. Dr. David P. Giedroc (sponsor and co-proposer of the present application) discovered a bacterial ABC transporter denoted EgtU in *Streptococcus pneumoniae (Spn)* that is highly specific for ET and is expressed in other Gram(+) human pathogens including *Enterococcus faecalis*, *Staphylococcus aureus*, and *Listeria monocytogenes.* These exciting findings open up many new questions as to how invading pathogens might leverage this molecule to resist host oxidative or other insults during infections.

In this new project, we propose structural, biochemical, and biophysical experiments designed to, in a medium-throughput “from gene to structure approach”, ***elucidate the structural and mechanistic basis of ET transport by EgtU homologues.*** Here, we propose biochemical, biophysical, and cryo-electron microscopy (cryo-EM) studies of the intact transporters solubilized in detergents and/or reconstituted in phospholipids, proteoliposomes or nanodiscs. These comprehensive studies will elucidate how ET is transported across the membrane to near-millimolar levels and will identify those structural features that distinguish EgtU from closely related transporters. These studies significantly extend existing biological data on *egtU* from diverse human pathogens. How and at what stage during an infection ET impacts the viability of pathogens provides a foundation for future studies of other human pathogens that encode EgtU, but which have lifestyles distinct from *Spn*.

SCIENTIFIC AREA WHERE THE PROJECT FITS BEST*

Life Sciences (LIF)