Name	LUIZ PEDRO CARVALHO
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2011

Career History

2000 – 2001: MSc student, Federal University of Rio Grande do Sul, BR
2001 – 2006: PhD student, Albert Einstein College of Medicine, US, With John S. Blanchard – Dept of Biochemistry
2006 – 2011: Postdoctoral fellow, Weill Cornell Medical College, US, With Carl Nathan – Dept of Microbiology and Immunology
2011 – 2015: Programme Leader Track National Institute for Medical Research, UK
2015 – 2017: Junior Group leader, The Francis Crick Institute
2017 –present: Senior Group Leader, The Francis Crick Institute

Major Awards, Honours and Prizes

2014: American Society for Microbiology – Merck Irving S. Sigal Memorial Award
2020: American Chemical Society Div. of Biological Chemistry/ACS Infectious Diseases Young Investigator Award

Membership of external committees, editorial boards, review panels, SABs etc

Review panels membership:
2020-2021: HCERES – Department of Microbiology, Institute Pasteur – site visit and review – France
2019-2020: HCERES – Center of Infection and Immunity of Lille, Institute Pasteur de Lille - site visit and review – France
2020-2024: UKRI - MRC Infections & Immunity Board
2020-current: Wellcome - M.B.C.F. Expert Review Group (*Ad hoc*)

SAB:
2014-current: Editorial Advisory Board – ACS Infectious Diseases

Lab Name	***Mycobacterial Metabolism and Antibiotic Research Laboratory***

Research programme and achievements

My lab aims to understand how major human pathogens, such as *Mycobacterium tuberculosis,* survive and subvert the host. Our scientific niche is the study of poorly or completely uncharacterised proteins (enzymes and transporters) and the metabolic pathways they belong to. These unannotated gene products or orphan proteins constitute a significant portion of the genome of *M. tuberculosis.* Lack of knowledge on what half of the genome actually does contributes to our superficial understanding of tuberculosis and to our inability to come up with novel antitubercular agents rapidly. In addition, orphan proteins constitute a really challenging area of research, in particular employing conventional approaches. We are also keen to understand how metabolites regulate metabolism via allosteric regulation of key metabolic enzymes. A methodological signature of our work is the pioneering application of liquid chromatography – mass spectrometry metabolomics to address these questions.

Within this quinquennial, we have successfully discovered previously unknown metabolites, enzymatic activities and metabolic pathways. In addition, we have mapped how nutrients are metabolised by *M. tuberculosis* and other bacteria, such as *E. coli* and *B. subtilis*. I would like to highlight: (i) the discovery of a truly redundant enzyme catalysing one of the key steps of gluconeogenesis in *M. tuberculosis*; (ii) the discovery of a mycobacterial non-canonical toxin which breaks down NAD^+. Instead of hydrolysis, this toxin uses inorganic phosphate and therefore it represents the first NAD^+ phosphorylase in nature; (iii) the first study of organic nitrogen acquisition/metabolism in *M. tuberculosis*, leading to a high-resolution blue-print of a bacterial nitrogen metabolic network highly evolved to operate inside a host (3); and (iv) the discovery of a bi-functional carbon-carbon bond lyase involved in host-itaconate resistance and L-leucine catabolism in *M. tuberculosis* (2). This work demonstrates that *M. tuberculosis* can grow on itaconate (a host-derived antibacterial molecule) as sole carbon source, instead of being killed by it, as had been assumed. Beyond *M. tuberculosis*, we contributed to an important study that demonstrates for the first time the central role of metabolism during the establishment of the L-form in Gram-positive bacteria. Outside of its inherent biological appeal, bacterial L-form protects bacteria against lysis mediated by β-lactam antibiotics.

In addition to our work on bacterial enzymology and metabolism, we have investigated how *M. tuberculosis* is killed by experimental compounds and by clinically approved antibiotics, most notably D-cycloserine (DCS). We have completely re-written the "textbook" mechanism of inhibition of DCS's targets, D-Ala:D-Ala ligase and alanine racemase. That is, DCS is phosphorylated in the active site of D-Ala:D-Ala ligase (4) and broken down on the active site of alanine racemase (1). We also investigated how resistance to DCS has not yet developed. This work demonstrated the importance of having two targets, which leads to an ultra-low frequency of resistance, and further showed that no-cost mutations leading to resistance can be developed. On the topic of phenotypic resistance to antibiotics, we have published an important study showing that the host milieu contains more NaCl than the media we use to study *M. tuberculosis*. This has profound effects on cell envelope composition and on antibiotic sensitivity against this bacterium.

In the next quinquennial period we intend to continue and expand our work on mycobacterial orphan genes. We will also continue to work on antibiotic mechanisms of action and resistance, particularly at the bacterial cell level. Finally, our work on allosteric regulation of metabolic enzymes by small molecules will likely be directed to other enzymes, as we have nearly exhausted the questions we wanted to answer with ATP phosphoribosyltransferase.

Research outputs

de Chiara C, Homšak M, Prosser GA, Douglas HL, Garza-Garcia A, Kelly G, Purkiss AG, Tate EW and de Carvalho LP. (2020) *D-Cycloserine destruction by alanine racemase and the limit of irreversible inhibition*. Nat Chem Biol 16(6):686. DOI: 10.1038/s41589-020-0498-9
D-cycloserine is an antibiotic used for decades to treat drug resistant tuberculosis. Its inhibition mechanism came into question when in a previous paper we determined alanine racemase activity in “fully inhibited” cells. This study demonstrated a previously unknown path during the assumed irreversible inhibition of alanine racemase that leads to the destruction of the antibiotic, meaning that alanine racemase is not irreversibly inhibited by the drug. The paper highlights the complexity of studying the chemical mechanisms of inhibition of enzymes and points to a novel strategy to design D-cycloserine analogues with improved properties.

Wang H, Fedorov AA, Fedorov EV, Hunt DM, Rodgers A, Douglas HL, Garza-Garcia A, Bonanno JB, Almo SC and Carvalho LP. (2019*) An essential bi-functional enzyme in Mycobacterium tuberculosis for itaconate dissimilation and leucine catabolism*. Proc Natl Acad Sci USA 116(32):15907. DOI: 10.1073/pnas.1906606116
Genes of unknown function represent one of the biggest biological challenges in the post-genomic era; in *M. tuberculosis*, we estimate that such genes constitute between 30 and 50% of the total. In this study we determined that a previously annoted member of the citrate lyase complex is in fact bifunctional. Instead of cleaving citrate, it catalyses the last step of itaconate catabolism and of leucine catabolism. Before this study, itaconate was thought to be toxic to *M. tuberculosis*, but we demonstrated that M. tuberculosis can actually grow on itaconate as sole carbon source.

Agapova A, Petridis M, Hunt DM, Garza-Garcia A, Sohaskey CD and de Carvalho LP. (2019) *Flexible nitrogen utilisation by the metabolic generalist Mycobacterium tuberculosis reveals a highly adapted metabolic network*. Elife 8: e41129. DOI: 10.7554/eLife.41129
Previously, we showed that *M. tuberculosis* uses host amino acids such as aspartate and asparagine as nitrogen sources during infection. Here we characterised quantitatively the use of amino acids as nitrogen sources by *M. tuberculosis*, and compared this to the use of ammonia, the dominant nitrogen source used by bacteria. Our results led to a fine mapping of the *M. tuberculosis* metabolic network, highilghting features that allow for the use of amino acids as nitrogen sources. Of note, M. tuberculosis has almost lost the ability to use ammonia, likely due to its evolution into a host-dependent species, where ammonia is scant.

Batson S, Chiara C, Majce V, Lloyd A, Gobec S, Rea D, Fulop V, Simmons K, Fishwick C, de Carvalho LP‡ and Roper DI‡. (2017) *Inhibition of D-Ala:D-Ala ligase through a novel phosphorylated antibiotic*. Nat Comm 8(1):1939. DOI: 10.1038/s41467-017-02118-7
D-cycloserine is an antibiotic used for decades to treat drug resistant tuberculosis. In addition to inhibiting alanine racemase, it inhibits D-Ala:D-Ala ligase. Together with the Roper group, we demonstrated that inhibition of the ligase is more complex, and D-cycloserine is phosphorylated in the active site of the enzyme, leading to a tighter inhibition. This mechanism was proposed decades ago, but never proved. Our results

provided additional structural and chemical proof for slow-onset kinetics, observed previously by our group.

Pisco JP, de Chiara C, Pacholarz KJ, Garza-Garcia A, Ogrodowicz RW, Walker PA, Barran PE, Smerdon SJ and de Carvalho LP. (2017) *Uncoupling conformational states from activity in an allosteric enzyme*. Nat Comm 8(1):203. DOI: 10.1038/s41467-017-00224-0

Ferredoxic-like domains have been used during the evolution of metabolic pathways to serve as small molecule sensor domains and allosteric regulators of key enzymes, from archaea to man. In this study we demonstrate in detail how inhibition via these domains works, by employing an activator in addition to the physiologic inhibitor. This activator was discovered using a novel strategy for small molecule screening. Finally, we demonstrated that the large conformational changes associated with regulation are in fact decoupled from inhibition. These results significantly expand our understanding of allosteric regulation of a number of metabolic pathways via these ferredoxin-like domains.