Functional studies of the human protein SQOR, identified as an interactor of the virulence factor PtpA of *Mycobacterium tuberculosis*.

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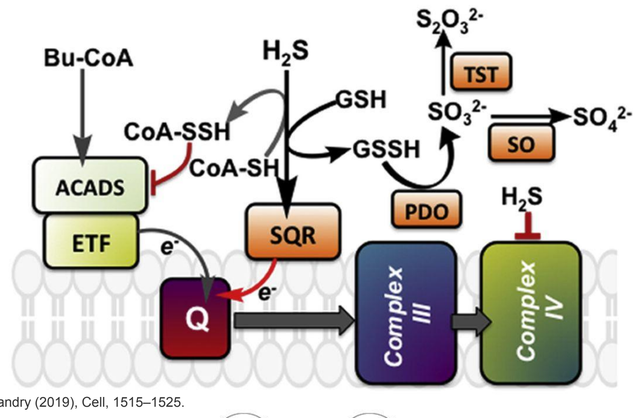
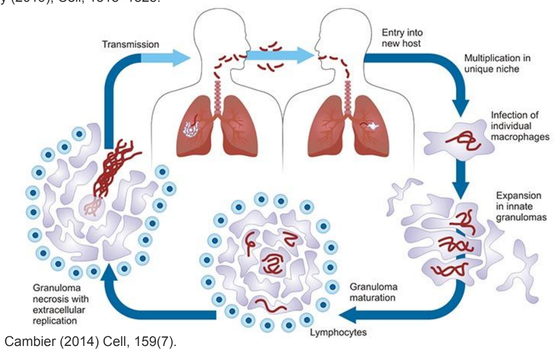
INTRODUCTION

Mycobacterium tuberculosis (*Mtb*) is the etiological agent of tuberculosis (TB), a disease that has caused the death of 30 million people worldwide in the last 20 years. *Mtb* can evade the immune response by surviving and replicating within macrophages, introducing various virulence factors into the cytosol. One of these is the tyrosine phosphatase PtpA, which is targeted to the macrophage cytosol during infection and is capable of modulating the innate immune response. Previous reports have shown that PtpA is able to interact with several proteins related to cell metabolism, such as sulfide quinone reductase (*h*SQOR). This cytosol-produced protein catalyzes H_2S oxidation in the mitochondria and is not detected in the organelle during virulent *Mtb* infection.



Landry (2019), Cell, 1515–1525.



Cambier (2014) Cell, 159(7).

MAIN OBJECTIVES

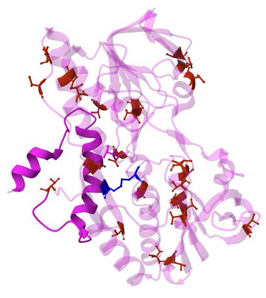
- Determine if *h*SQOR is a substrate of the mycobacterial phosphatase PtpA
- Evaluation of the role of Tyr phosphorylation on the activity of *h*SQOR variants
- Study of the *in cellulo* interaction of *h*SQOR with wild type and ΔPtpA *Mycobacterium bovis* BCG

METHODOLOGY AND PRELIMINARY RESULTS

➢ Evaluation of exposed p-Tyr of *h*SQOR and its interaction with PtpA active site

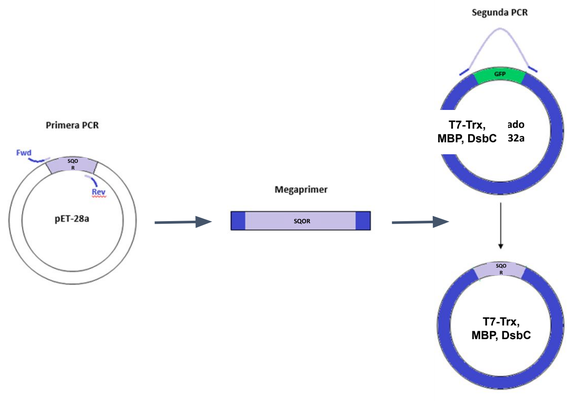
mPtpA 1U2P

*h*SQOR 6OI5

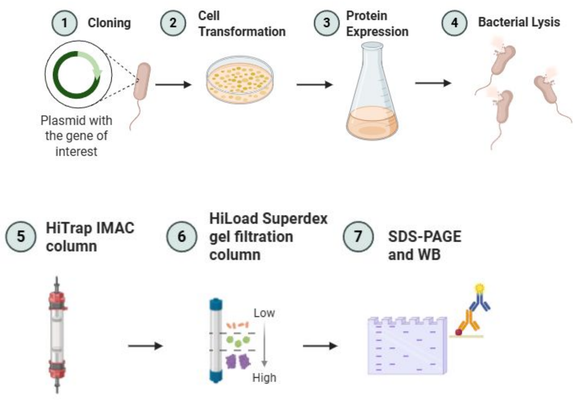


HADDOCK High-Ambiguity Driven Docking

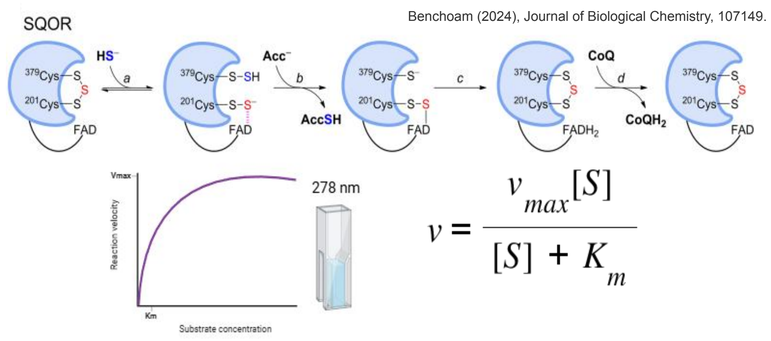
➢ RF cloning to generate: T7-Trx-GFP, T7-MBP-GFP and T7-DsbC-GFP



➢ Expression and purification of the recombinant *h*SQOR proteins

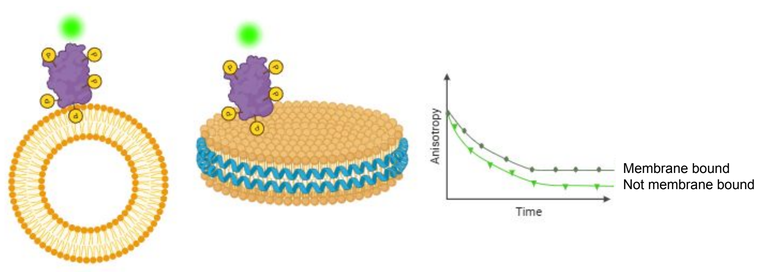


➢ Role of pTyr in *h*SQOR variants activity

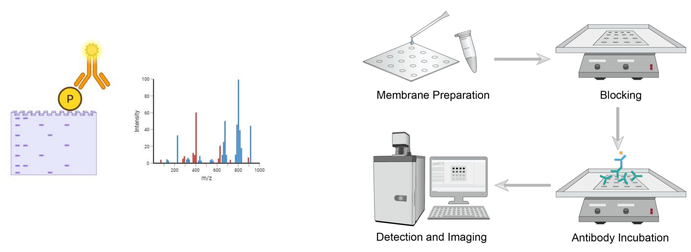


Benchoam (2024), Journal of Biological Chemistry, 107149.

➢ Phosphorylation effect on the ability of *h*SQOR variants to bind to artificial membrane models

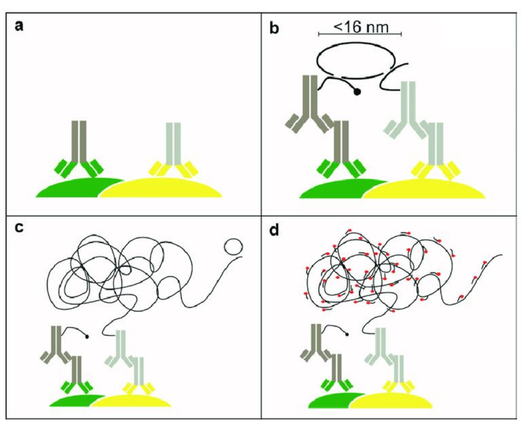


➢ Evaluation of *h*SQOR phosphorylation status and as a substrate of PtpA



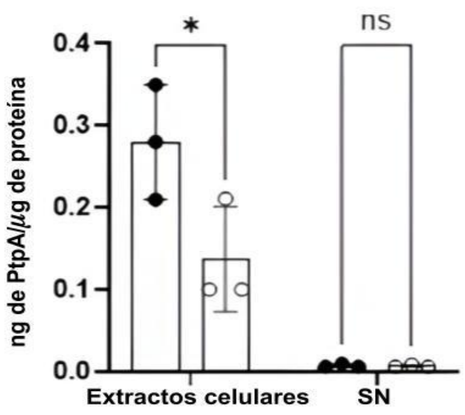
(or ab65622 Phosphate Assay Kit)

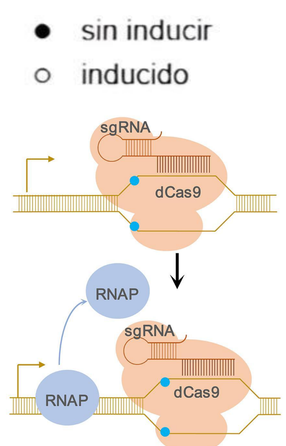
➢ Interaction between PtpA and *h*SQOR during infection with Duolink PLA (Sigma)



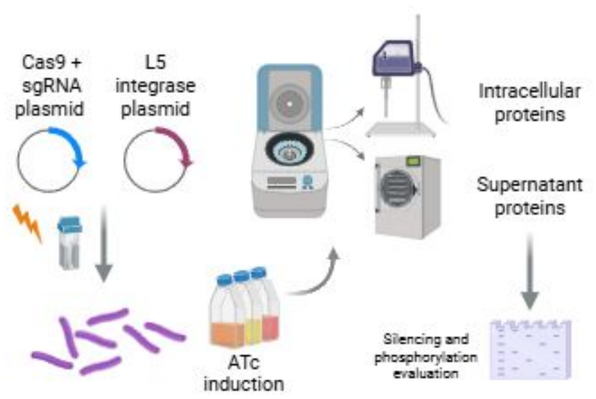
Trifilieff (2011), Biotechniques, 111-8.

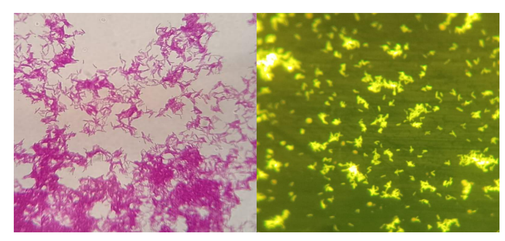
ΔPtpA mutants were developed in *Mycobacterium smegmatis* with CRISPR/Cas9i strategy in my graduate thesis





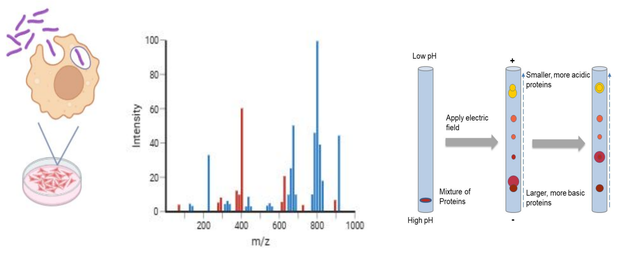
➢ ΔPtpA mutants in *Mycobacterium bovis* BCG

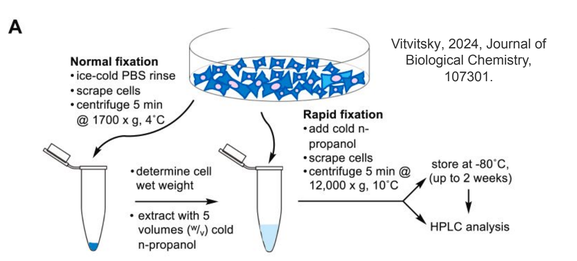




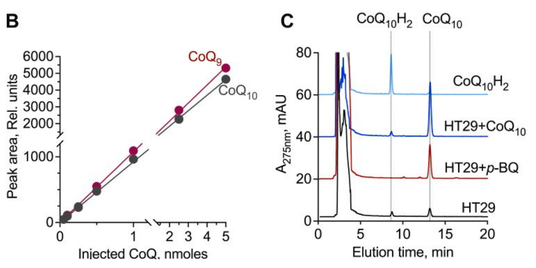
Ziehl-Neelsen and Auramine O staining of *Mycobacterium bovis* BCG cultures

➢ Phosphorylation state of *h*SQOR and CoQred/CoQox ratio in cellular extracts after infection

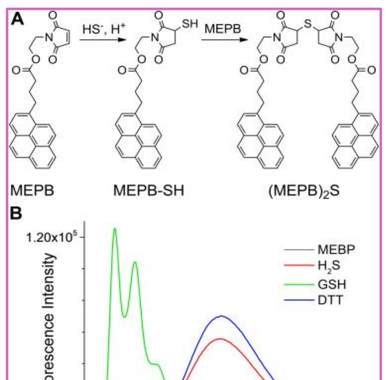


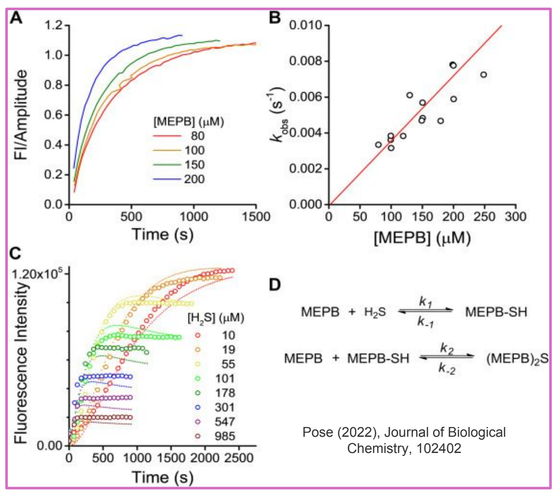


Vitvitsky, 2024, Journal of Biological Chemistry, 107301.

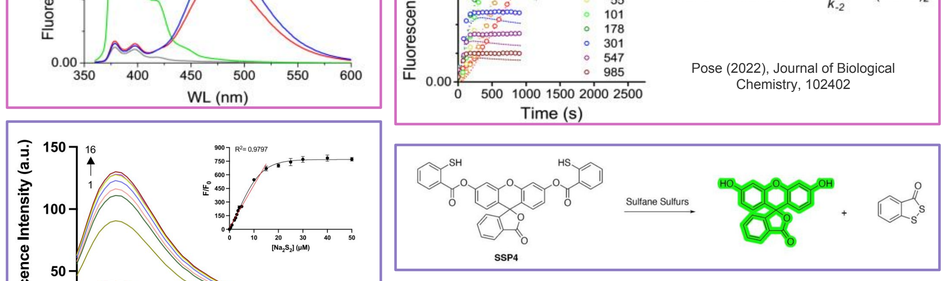


➢ Determination of H_2S and sulfane sulfur compounds concentration in culture supernatant



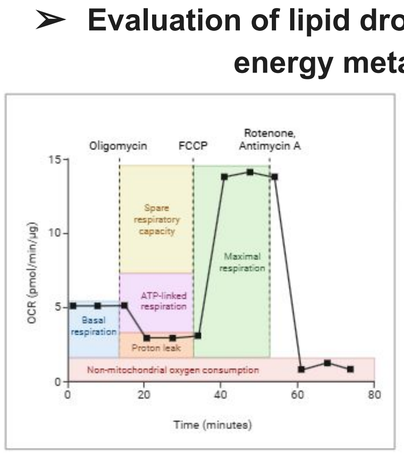


Pose (2022), Journal of Biological Chemistry, 102402

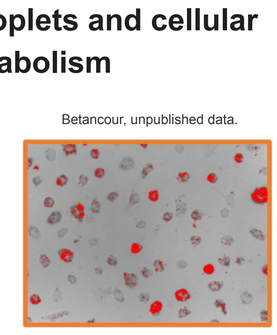


Shieh, 2022, Redox biology, 102433.

➢ Evaluation of lipid droplets and cellular energy metabolism



Betancour, unpublished data.



Oil red O staining of infected macrophage cultures

ACKNOWLEDGEMENTS

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ANII AGENCIA NACIONAL DE INVESTIGACIÓN E INNOVACIÓN