Investigating the escape of *Mycobacterium tuberculosis*

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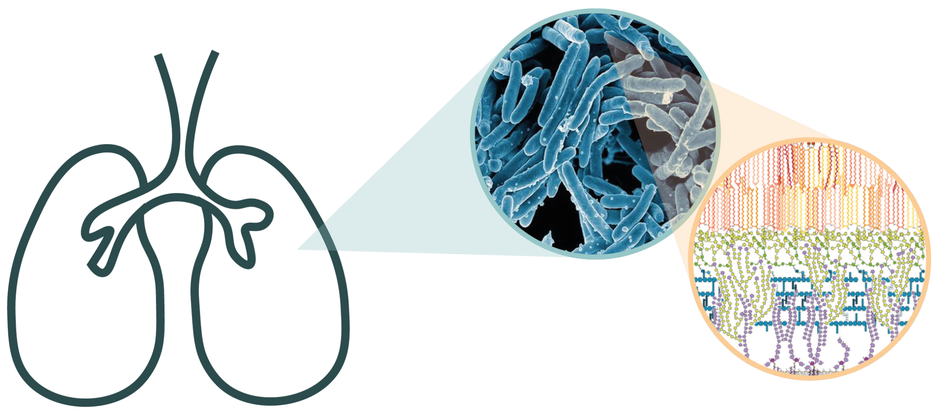


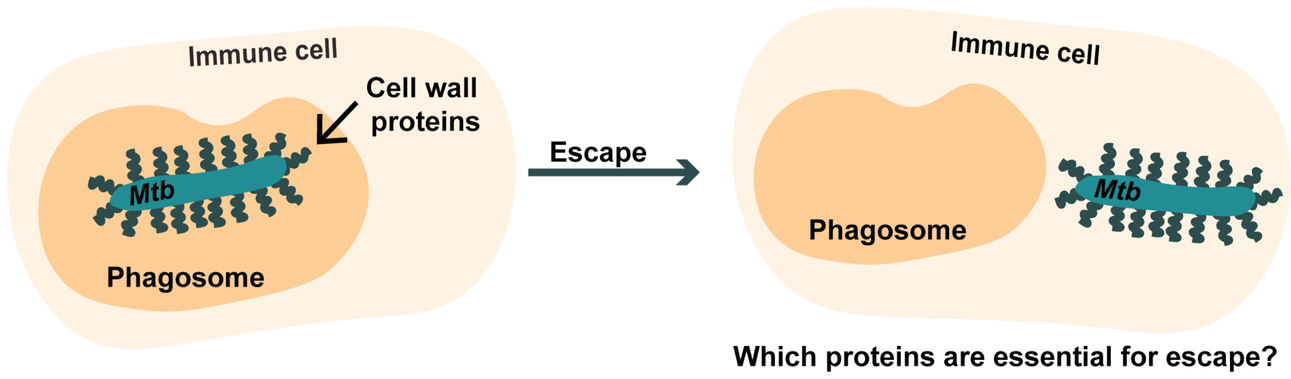
Mycobacterium tuberculosis

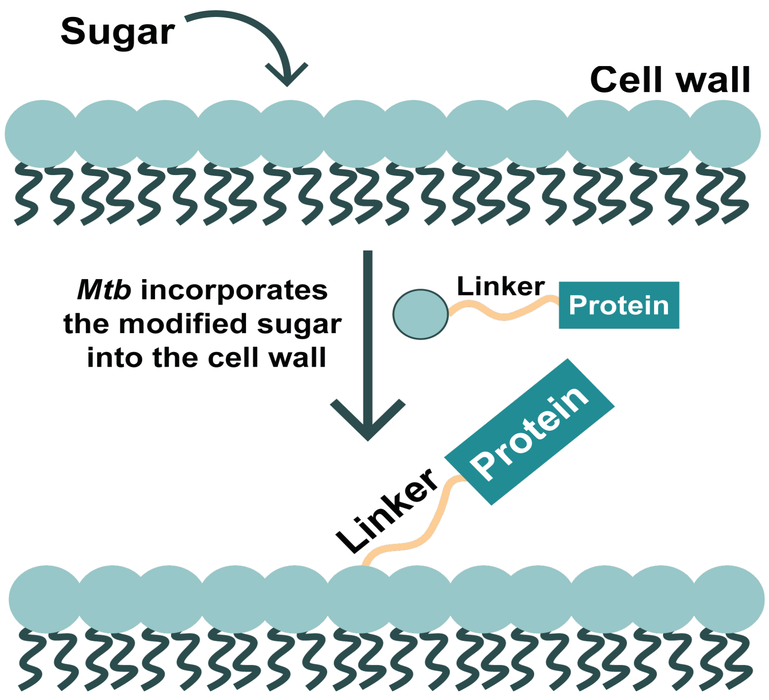
Tuberculosis is the worlds most deadly bacterial infectious disease, and mostly affects the **lungs**. [1] It is caused by ***Mycobacterium tuberculosis (Mtb)***. Treatment involves intensive antibiotics regimen for 6-24 months. However, antibiotic resistance is becoming a serious problem.

Mtb is a good pathogen due to its special **cell wall**, which protects it against antibiotics. [2] Furthermore, the cell wall helps the bacterium to survive the degradation mechanisms of the human body. This way, *Mtb* can survive in the human body for years.

Phagosomal escape

One of the main mechanisms for the human body to protect itself against pathogens is the generation of a phagosome, an acidic 'stomach', to degrade pathogens. [3] *Mtb* has developed mechansms to avoid this degradation. The most important is its ability to escape the phagosome and survive in the less hostile immune cell. [4] It is known that proteins on the cell wall contribute to this **phagosomal escape**. However, the exact proteins required for this are still unknown.





Goals

We aim to investigate which proteins are involved in the escape process. This will help towards:

(1) **Understanding** the survival mechanisms of *Mtb*

(2) **Discovering** new targets for medicines and vaccines

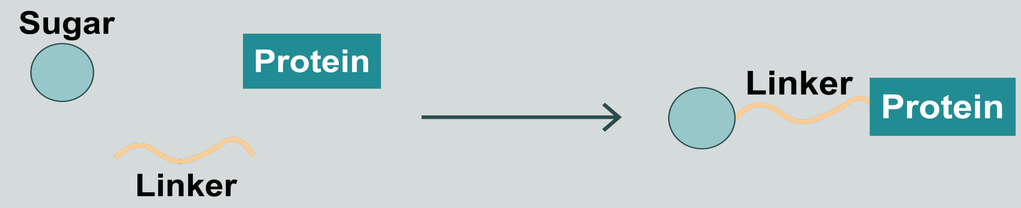
We designed a method that allows for selective incorporation of the putative 'escape proteins' into the cell wall of *Mtb*, using the endogenous metabolism of the cell. This allows to incorporate a sugar-linker-protein structure into the mycobacterial cell wall. Our goals were:

(1) Development of a **fast and universal synthesis** method for the sugar-protein complex

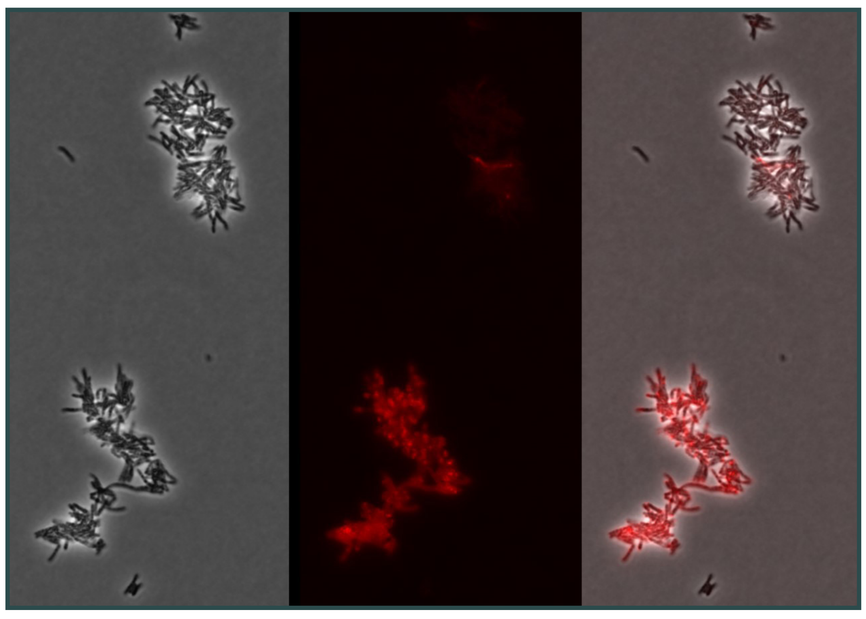
(2) **Proof of concept** using a fluorescent protein

Results

We developed and optimized a procedure for the sugar-protein constructs. Both synthesis and analysis are **easy and fast**. In our **tunable** design, the linker can easily be adapted. And lastly, the sugar-linker-protein constructs can be made for a **wide variety of proteins**.



With the synthesis method in hand, we performed initial incorporation experiments using a **red-fluorescent protein**. It was shown that the construct is incorporated!



Outlook

With the proof of concept in hand, we aim to **optimize the protein incorporation** into the mycobacterial cell wall. Subsequently, we plan to incorporate the escape proteins and investigate their role in the process.

References

[1] Global Tuberculosis Report **2021**, World Health Organisation. [2] V. Jarher *et al.*, "Mycobacterial cell wall: Structure and role in natural resistance to antibiotics," FEMS Microbiol. Lett. **1994,** 123, 11–18 [3] K. Rohde *et al.*, "*M. tuberculosis* and the environment within the phagosome," Immunol. Rev. **2007**, 219, 37–54. [4] N. van der Wel *et al.*, "*M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells," Cell **2007**, 129, 1287–1298.

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