Mycobacterium tuberculosis is the causative agent of human tuberculosis (TB) and can be considered one of the most efficient pathogens in history, as it has threatened our health since the beginning of Homo sapiens' existence [1]. Nowadays, it is estimated that one-quarter of the world’s population has latent TB, from which 10 million people fell ill in 2018. Every year, 1.5 million people succumb to TB, placing it together with COVID-19, as the leading cause of death from a single infectious agent [2]. Although TB chemotherapy is considered a triumph of anti-infective research, changing the disease from fatal to curable is far from optimal. The toxicity of the drugs and the length of the treatment have contributed to the rise of drug-resistant strains that threaten global health security [3]. The need for a new medicine that can cure or prevent TB is unquestionable, but gaps in the knowledge of mycobacteria pathogenesis hampers its development. Additionally, M. tuberculosis has diverged into several phylogenetic lineages with different virulence degrees hindering our further understanding of the molecular mechanisms of the pathogenesis. Nevertheless, there are essential events that contribute to the infectivity success of all pathogenic strains. As part of the innate immune system and the first line of defence against pathogens, macrophages internalise the bacteria in a process called phagocytosis with the intention to degrade the microorganism. However, M. tuberculosis and other pathogenic species evade this fate by blocking the maturation of the phagosome and disrupting the phagosomal membrane to translocate into the host cell's cytosol [5]. This event is essential for the survival of mycobacteria, as it has been shown that non-pathogenic species are unable to translocate, leading to the lysis of the bacteria. This ability has been directly linked to the presence of the ESX-1 secretion system [6]. We will present a structural study (Fig. 1), by small angle X-ray scattering (SAXS) in combination with other structural biology techniques, secretion-associated substrate EspB bound to its chaperone EspK [7-8], both from M. tuberculosis. The main findings include, revealing the presence of two well-defined domains in EspK connected by an unstructured, low complexity linker. Likewise, in combination with cryo-EM and X-ray crystallography, SAXS showed that EspK C-terminal domain interacts with EspB in its monomeric form, acting as a chaperone by preventing premature oligomerization. The study opens a new approach to a rational drug design for TB.

**Keywords:** SAXS, tuberculosis, EspK-EspB

**Figure 1.** a) SAXS on EspK full length structure, b) SEC-SAXS experiments show no EspB oligomer in the presence EspK.


