Unravelling the Structural Mechanism of the Pneumococcal Manganese Transporter PsaBCA

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Streptococcus pneumoniae is a Gram-positive bacterial pathogen of major clinical importance, responsible for more than one million deaths annually. The acquisition of transition metals such as manganese (Mn) from the human host is crucial for its survival and virulence. *S. pneumoniae* primarily obtains trace metals through ATP-binding cassette (ABC) transporters, and disruption of manganese uptake significantly attenuates its pathogenicity. Despite their critical role in bacterial virulence and absence from the human genome, the molecular mechanisms underlying metal recognition and transport by these systems remain poorly understood. Elucidating these processes could provide valuable insights for the development of novel antimicrobial therapeutics. Manganese acquisition in *S. pneumoniae* is mediated by the ABC transporter PsaBC in complex with the solute-binding protein (SBP) PsaA. Our group previously determined the crystal structure of the apo form of PsaBC the first cation-translocating ABC transporter in an open-inward conformation. However, the mechanism of manganese import, including the structural transitions associated with ATP binding and hydrolysis, as well as the interaction between PsaA and PsaBC, remain unresolved. We aim to characterize the structural transitions of the PsaBCA complex during manganese transport using cryo-electron microscopy (cryo-EM). To date, we have successfully purified the apo PsaBC complex in Cymal-6, and ongoing sample optimization efforts are focused on obtaining higher-resolution structures of the apo form and the nucleotide-bound form using non-hydrolysable ATP analogs. Preliminary results indicate that the PsaBCA complex is transient; therefore, glutaraldehyde cross-linking will be employed to stabilize the full complex for structural analysis.