Staphylococcus aureus is a major human pathogen that has acquired an alarming broad-spectrum resistance to many of the commonly used antibiotics including beta-lactams such as penicillin. S. aureus often causes hospital- and community- associated infections responsible for significant morbidity and death. Staphylococci infections are mediated through a large array of secreted toxins including the phenol-soluble modulins (PSMs). PSMs are amphipathic, α-helical peptides with pronounced surfactant-like properties that have multiple key roles in pathogenesis, including cytolysis of red and white blood cells, abscess formation, biofilm development and trigger receptor-mediated inflammatory response. A specialized ATP-binding cassette (ABC) transport system exports PSMs to the extracellular environment and is essential for bacterial growth by providing an immunity against self-expressed PSMs. Here, we present the structural characterization of the PSM transporter determined by high-resolution single-particle cryo-EM and X-ray crystallography accompanied with functional characterization in vivo. The observed alternations between different transport stages provide crucial mechanistic insights and sets the foundation for novel therapeutics design.