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Studying structure and function of sialic acid TRAP transporters from pathogenic bacteria by pulsed EPR, FRET and X-ray crystallography

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Many pathogens such as *Vibrio cholerae* use tripartite ATP-independent periplasmic (TRAP) transporters to scavenge N-acetyl- neuraminic acid (sialic acid) from host organisms. The sialic acid is then incorporated into the bacterial cell wall, as a disguise to protect against detection by the human immune system. TRAP transporters are a structural and functional mix between ABC transporters and secondary active transporters. The substrate binding proteins (SBP) of TRAP transporters are the best studied component and are responsible for initial high-affinity substrate binding. To better understand the dynamics of the ligand binding process, pulsed electron-electron double resonance (PELDOR, also known as DEER) spectroscopy and FRET were applied to study the conformational changes in the N-acetylneuraminic acid-specific SBP VcSiaP. The protein is the SBP of VcSiaPQM, a sialic acid TRAP transporter from *Vibrio cholerae*. Spin-labeled double-cysteine mutants of VcSiaP were analyzed in the substrate-bound and -free state and the measured distances were compared to crystal structures of the labelled protein. The data were compatible with two clear states only, which are consistent with the open and closed forms seen in TRAP SBP crystal structures. Substrate titration experiments demonstrated the transition of the population from one state to the other with no other observed forms. Mutants of key residues involved in ligand binding and/or proposed to be involved in domain closure were produced and the corresponding PELDOR experiments reveal important insights into the open-closed transition. Further, PELDOR distance measurements on the whole transporter in lipid nano discs were used to evaluate molecular models of the transporter.

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