Substrate-dependent binding of MalE to the type I ABC transporter MalFGK$_2$.

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The ubiquitous ABC-transporter family is involved in the ATP-dependent uptake or export of a large variety of substrates. ABC-transporters share two conserved nucleotide-binding domains and two substrate-specific transmembrane domains. In the case of importers, a specific substrate-binding protein exists, which captures the substrate in the periplasm and delivers it to the importer. The type I importers have a low-affinity ($K_D$ in the upper micromolar range) [1] to the substrate binding protein (SBP), whilst the affinity of the SBP to the type II importers is in the nM range, highlighting their mechanistic diversity.

The maltose import system is structurally and biochemically well characterized and two crystal structures of the transporter are available in two different nucleotide states: a low resolution apo-state in the absence of MalE (PDB: 3FH6) [2] and a high resolution ATP-bound state in the presence of MalE and bound maltose (PDB: 2R6G) [3]. The role of the periplasmic P2-loop of MalFGK$_2$ in the overall import mechanism and the communication between the substrate-loaded MalE to the transmembrane domain is still unclear. The aim of this study is to characterize the formation of the complex between the maltose-binding protein MalE and MalFGK$_2$ from E.coli during the nucleotide cycle in the presence and absence of maltose, to complement the available structural data and to obtain new insights in the role of the substrate in the mechanism of import.

Our method of choice is site directed spin labeling (SDSL) in combination with double electron electron resonance (DEER) to obtain precise distance information between selectively labeled sites. The results clearly show that MalE interacts with the transporter in all states of the nucleotide cycle, independently on the presence of maltose. However, the MalE-MalFGK$_2$ interaction in the apo state takes place mostly between the N-lobe of MalE and the P2-loop of MalFGK$_2$ in the absence of maltose, whereas in the presence of maltose MalE binds via both the N- and C-lobe to the transporter. In contrast, the conformation of the MalFGK$_2$-E complex in the ATP-state is only driven by binding of ATP, regardless of the presence or absence of maltose. The EPR results are compared to the maltose-dependent activity of the transporter and a more complete model of the substrate import cycle of the type I ABC importer is presented.