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**Structure-Based Engineering of Maltose Binding Protein.** P.G. Telmer, B.H. Shilton, Univ. of Western Ontario, London, Canada.

Maltose Binding Protein (MBP) is the primary receptor for uptake of maltose by the maltose transporter of *E.coli*. MBP undergoes a ligand-induced conformational change, which signals the initiation of transport across the membrane, and is required for transport to occur *in vivo*. We have taken a protein-engineering approach to study MBP's role in the conformational activation of the membrane complex, as well as its ability to regulate substrate specificity. Removal of interactions within a balancing interface opposite the maltose binding cleft, destabilized the open state of MBP, leading to altered substrate affinity. Structural and kinetic studies indicated that these mutations altered the open-closed equilibrium of MBP during substrate binding without altering the structural endpoints. We have also constructed and purified previously described MBP mutants (Marvin et al. *PNAS*. 2001), which can coordinate metal ions within MBP's substrate binding site. Crystal structures of metal-binding mutants indicate that the altered substrate specificity has unexpected effects on the conformation of the metal-bound molecule. Since the specific conformation of MBP both in the presence and absence of ligand is important for the activation of the transport machinery, structural studies of substrate-altered molecules will aid in our understanding of this activation.