Mhp1 from *Microbacterium liquefaciens* is a member of the Nucleobase-Cation-Symport-1 (NCS-1) family of membrane transport proteins that uses a sodium gradient to energise the uptake of hydantoins. We recently elucidated the 3d X-ray crystal structures of Mhp1 in three conformations – outward-open, occluded, and inward-open – that revealed the structural basis of its ‘alternating access’ mechanism. Mhp1 shows unexpected structural and mechanistic homology to the mammalian neurotransmitter transporter homologue LeuT, the Na⁺- galactose symporter vSGLT, the Na⁺-betaine symporter BetP, and the proton-coupled amino acid transporter, ApcT. Further elucidation of the substrate binding and transport mechanisms in Mhp1 is therefore of broad significance.

Site directed mutagenesis was undertaken to investigate putatively key residues in the substrate binding and transport mechanisms of Mhp1. Hydantoin binding was monitored by changes in the intrinsic tryptophan fluorescence. Mutagenesis of selected amino acids has caused changes in the substrate affinity, sodium dependence and radiolabelled hydantoin transport in Mhp1, which illuminate its molecular mechanism.