A residue of the Leu/Phe-valve in the extracellular gate directly and allosterically modulates Arabidopsis ABCG36 substrate specificity

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G-type ATP-binding cassette (ABC) transporters feature a hydrophobic "di-leucine motif" in their extracellular gate, which separates a large substrate-binding cavity from a smaller upper cavity and functions as a valve regulating substrate extrusion.
In this study, we demonstrate that the L704F mutation within the extracellular gate of Arabidopsis ABCG36/PDR8/PEN3 uncouples the transport of the auxin precursor indole-3-butyric acid (IBA) from the defense compound camalexin (CLX). Notably, L704 mutations to Ser and Tyr result in gain-of-function, enabling the transport of the ABCG36 non-substrates IAA and indole. Using advanced molecular dynamics simulations, including metadynamics and pulling simulations, we explored the effects of these mutations and identified key residues involved in substrate recognition and transport. Our findings reveal that interactions between substrates and residues on the cytosolic side of the central binding pocket negatively affect transport. Furthermore, residue L704 influences the transport process both directly and indirectly by modulating the allosteric coupling between the extracellular gate and the central binding pocket.
In conclusion, our study highlights L704 as a critical residue of the extracellular gate that governs substrate specificity by acting as a final quality control step in the transport process, thus advancing our understanding of substrate recognition and specificity in ABCG transporters.