

# Determinants of cargo specificity in glucosinolate transporters belonging to the nitrate and peptide transport family

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In plants, transport processes across cellular membranes are intricately involved in controlling the spatiotemporal distribution of a plethora of specialized metabolites with essential roles for plants. While several of the associated transporter families are known, the molecular bases underlying their ability to recognize cargo among the enormous structural diversity of these compounds remains elusive. Here, through cryogenic-electron microscopy, rational cargo-preference-swapping and molecular modelling, we investigated two members of the nitrate and peptide transporter family (NPF), GTR1 and GTR3. These proteins are essential for transporting so-called glucosinolate (GLS) defense compounds in *Brassicales* plants such as *A. thaliana*. Our study identifies the overall structure in two distinct states, including key residues determining binding pocket architecture and cargo specificity. Notably, amino acids crucial for activity reside in the middle or towards the 'inside' in this central void, while those determining GLS-specificity are located further toward the 'outside', suggesting how the cargo is oriented. Moreover, in the 'outward'-facing partially occluded state we identify a narrow pathway that may serve to facilitate uptake of co-transported protons from the 'outside'. This protonation process may be critical for allowing binding of GLS, explaining the electrogenic nature of transport of GTRs. Collectively, our findings offer a molecular understanding of GLS transport, and pave the way for further in-depth studies in basic and applied sciences to fully decipher the transport principles, and to modulate the transport capabilities of GLS in plants for biotechnological purposes.