Title: *Lock-and-Key Substrate Recognition in the Multidrug ABC Transporter Pdr5 from Saccharomyces cerevisiae***

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Abstract:

The ATP-binding cassette (ABC) transporter Pdr5 from *Saccharomyces cerevisiae* is one of the most abundant plasma membrane ABC transporter and a central component of the pleiotropic drug resistance (PDR) network, which protects cells from a wide range of xenobiotic compounds, including azoles, ionophores, antibiotics, and other toxic chemicals. Pdr5 has become an established model for fungal PDR transporters, many of which contribute to multidrug resistance in pathogenic fungi. Despite decades of research, the molecular basis underlying Pdr5's remarkable substrate promiscuity has remained elusive.

Here, we present four high-resolution cryo-electron microscopy (cryo-EM) structures of Pdr5 in complex with four chemically diverse substrates: the azole antifungals ketoconazole and fluconazole, the protein synthesis inhibitor cycloheximide, and the imidazole derivative clotrimazole. Strikingly, all four structures reveal an exceptionally conserved substrate-binding pocket that undergoes minimal conformational changes upon substrate binding. This observation contrasts with the induced-fit model commonly proposed for multidrug transporters and instead highlights a "lock-and-key" mechanism of substrate recognition. The rigid cavity of Pdr5 accommodates chemically distinct compounds primarily through conserved interactions, suggesting that structural rigidity—rather than flexibility—underlies its ability to recognize a broad spectrum of substrates.
Together, these findings provide new mechanistic insights into substrate recognition by Pdr5. This work enhances our understanding of multidrug resistance in fungi and establishes a foundation for future studies targeting PDR transporters in pathogenic species.