Understanding Bacterial Resistance Mechanisms: Insights from Porin Mutations in Klebsiella pneumoniae

Matteo Ceccarelli

Department of Physics, University of Cagliari, Italy

Small polar molecules are currently a primary line of defense against bacteria. They can be highly selective for bacterial cells, resulting in reduced toxicity, and can be identified through screening synthetic compound libraries, which offer an almost infinite number of potential molecules for discovering new inhibitors and antibiotics. Bacterial porins—nanometric pores expressed in Gram-negative bacteria—serve as the main pathway for small polar molecules to traverse the outer membrane and reach internal targets.

Although the role of porins in bacterial resistance was postulated in the 1980s, there were few well-described examples in the literature. In recent years, several research groups have focused on *Klebsiella pneumoniae*, one of the most pathogenic and resistant Gram-negative organisms. Among resistant strains, mutations have been identified that clearly point to amino acid substitutions in a specific region of the OmpK36 porin, which is highly expressed during infections.

High-resolution X-ray structures of the OmpK36 pore revealed that these mutations appear to affect pore size rather than electrostatic properties, as previously believed. However, X-ray diffraction structures are inherently static and do not capture the physiological conditions of ion concentrations and temperature. Only through molecular simulations can we precisely determine the size and flexibility of the pore under physiological conditions. We characterized the wild-type OmpK36 and a series of three resistant porins by calculating pore size fluctuations and electrostatic properties using enhanced sampling molecular dynamics simulations, see Fig.1.

By comparing the distribution of pore sizes and the strength of the internal electric field, we determined the effect of point mutations on the permeability of small molecules. Using our predictive model for permeability, we quantified the permeability of different antibiotic molecules through the characterized porins. Our data, combined with published experimental data on susceptibility, allowed us to establish a threshold at which reduced permeation begins to impact resistance.

Our results contribute to understanding how bacterial resistance evolves, providing crucial insights for the design of new molecules to counteract bacterial infections. Additionally, they offer a quantifiable minimum permeability required for an antibiotic to be effective, providing a threshold for screening virtual libraries of related compounds.

Figure 1: Comparative analysis of OmpK36 with selected mutants for [A] distribution of internal minimum radius, and [B] internal transversal electric field.