A bottom-up approach for rationalising molecule permeation in Gram-negative bacteria.

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The demand of new drugs for combating multidrug-resistant bacteria appears more urgent for Gram-negative bacteria: the presence of the outer membrane, which hinders the access of molecules to internal targets, renders the development of anti-infectives more challenging. Today neither a robust screening method for permeation nor defined physical/chemical rules governing permeation through the outer membrane are available.

As part of the IMI-ND4BB platform, a Public-Private Partnership (PPP) funded by the EU and EFPIA, the Translocation project is applying a bottom-up approach to investigate the problem of molecule permeation through the outer membrane. We started from high-resolution crystal structures of simple and gated porins, followed by monitoring their expression in the bacteria in different growth conditions. With this information we defined a priority list of proteins and selected antibiotics and inhibitors from different families to rationally explore the chemical space (charge, size, shape). We then combined all-atom molecular modeling with molecular dynamics enhanced sampling techniques to characterize electrostatic of porins and simulate the permeation of antibiotics through porins, identifying the key molecular parameters.

We focused initially on Enterobacteriaceae, where OmpF/OmpC orthologs constitute the main influx routes for polar antibiotics. The results were analyzed with a simple Hamiltonian model, showing how (i) the minimal projection area and (ii) the molecular dipole moment represent key parameters to modulate permeation through simple porins. Other parameters such as size/shape fluctuations, which correlate with rotatable bonds, were also considered. Cell-free electrophysiology at single molecule level on reconstituted porins was used to confirm our data whenever possible.

Without a strong and accurate experimental method to assess the flux of antibiotics through porins, our Hamiltonian model, based on steric hindrance and electrostatic of pore/antibiotic complex, represents a useful tool to explore the chemical space for searching new scaffolds and/or optimize at the molecular level existing molecule for an enhanced permeation through bacterial porins.

Acosta-Gutierrez, et al. (2015). Filtering with Electric Field: The Case of E. coli Porins. *Journal of Physical Chemistry Letters*, *6*, 1807–1812. http://doi.org/10.1021/acs.jpclett.5b00612

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Winterhalter & Ceccarelli (2015), Physical methods to quantify small antibiotic molecules uptake into Gram-negative bacteria. *Eur. J. Parm. Biopharm.* 95:63-7.