Predicting permeabilities of small-molecules through bacterial porins: the scoring function 2.0.

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The outer membrane of Gram negative species hinders the access of hydrophilic molecules to internal targets, making the development of anti-infectives particularly challenging. Several investigations pointed out that outer membrane porins represent the main entry path for small antibiotics in the periplasmic space. Few years ago, we measured permeabilities of 9 antibiotics through 8 porins from *Enterobacteriaceae* with liposome swelling assays, and we demonstrated that permeation can be predicted with a scoring function. Besides providing a functional form for the prediction of the permeability, which is based on simple properties of molecules (size, charge, dipole) and pores (size and electrostatic properties), we demonstrated that all general porins from Enterobacteriaceae possess the same filtering mechanism, though differences in size and electrostatic. With our scoring function, we predicted the permeation of a large set of polar non-antibiotic molecules for which accumulation data in *Escherichia coli* were available from the literature, obtaining a large correlation, see figure.

With the aim to improve the predictability of our scoring function and make it more versatile, we recently planned a new campaign of measurements and the use of machine learning tools. We focused our liposome swelling assays on two porins, OmpF and OmpC, for which we calculated new parameters obtained by applying enhanced sampling techniques in numerical simulations. Then, we selected a larger set of molecules, a training set of 40 molecules and a test set of 27 molecules. The measured relative permeability coefficients, combined with a machine learning approach, allowed us to reparametrize the original scoring function. In the future, we plan to apply the same protocol to porins from *Acinetobacter baumannii*.

The new scoring function is more robust and its application can now start directly from the SMILE, obtaining the predicted permeability without external intervention. It can be applied either to small set of selected compounds and to large virtual libraries, with the aim to accelerate the optimization of new leads and to search for new scaffolds with optimal permeation, respectively. It represents a key and cheap predicting tool in the early stage drug discovery process.