SUPPLEMENTARY INFORMATION

The Role of Water during the Extrusion of Substrates by the Efflux Transporter AcrB

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Analysis Methods

Throughout the entire article, several nonstandard methods are employed to describe and analyse the extrusion process in molecular detail. All of these methods have been implemented in VMD (Humphrey, W.; Dalke, A.; Schulten, K. *J. Mol. Graph.* **1996**, *14*, 33–38).

Grid Path for Direction of Water Movement

The grid path (see Figs. 7, 8, and 10) is modeled from the entrance between the subdomains PC1 and PC2, here simply calculated as the COM of these domains, via the COM of the waters surrounding the BP to the COM of the exit gate. As there are only few waters in the BP, the point of measurement at the BP was shifted to the bordering waters (the selected waters are in a region of 3.5 Å from the COM of the BP) because we are more interested in the movement of internal waters in general. The grid path is divided into five equidistant segments along both parts, i.e., five segments from the entrance to the BP and another five from the BP to the exit resulting in eleven grid points. At each of these grid points, waters within a sphere of 9 Å radius are selected. Then the distance each water molecule moves within 50 ps is measured. At the end of the procedure, those displacements are averaged per grid point leading to average directed displacements per 5 ps per water molecule.

Additional Graphs

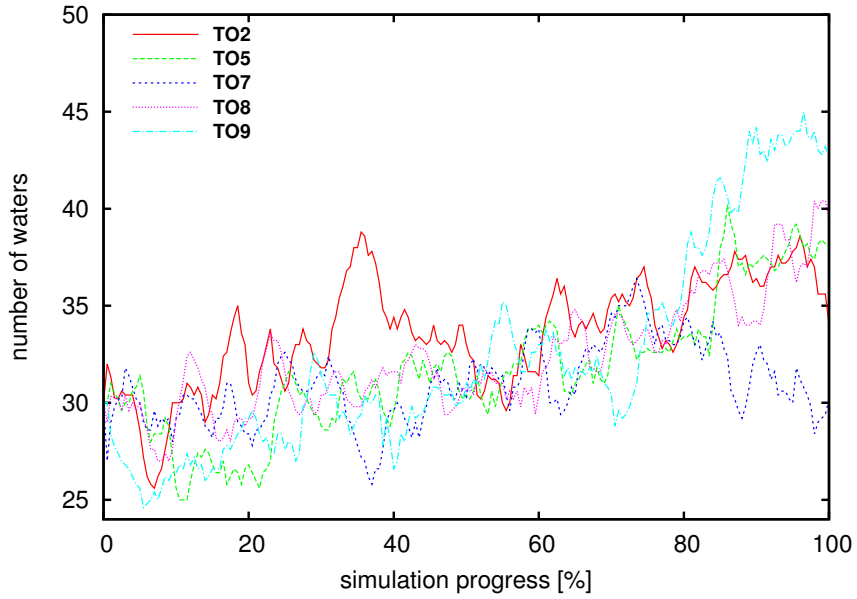

Figure S1: Number of water molecules surrounding the substrate as a function of time for representative TO trajectories.

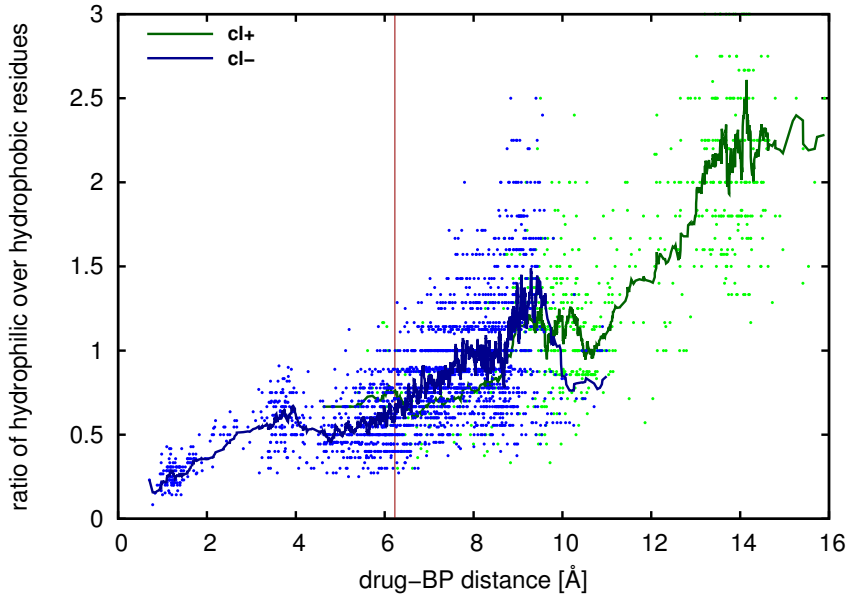

Figure S2: Ratio of hydrophilic over hydrophobic amino acids in the vicinity (3.5 Å) of the substrate as a function of the drug-BP distance of all TO simulations including a running average.

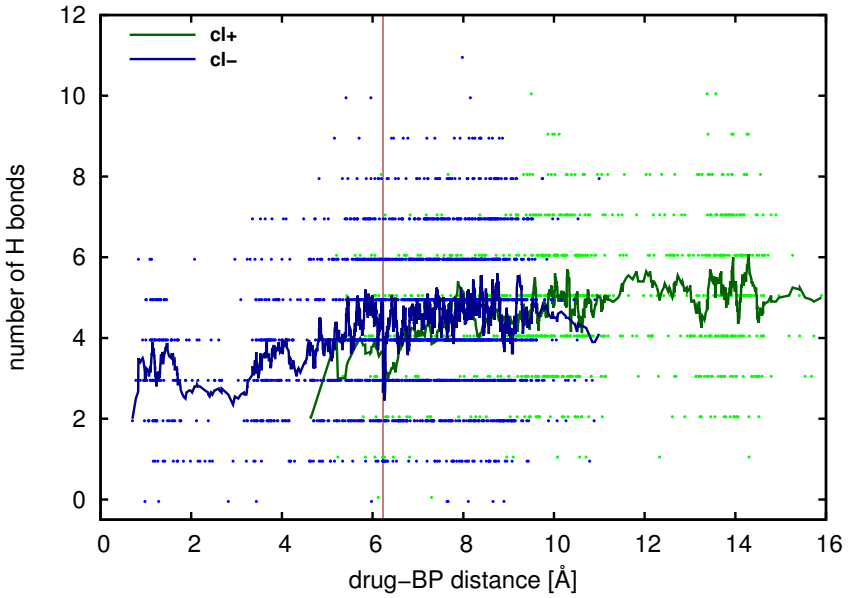

Figure S3: Number of hydrogen bonds as a function of the drug-BP distance of all TO simulations including a running average.

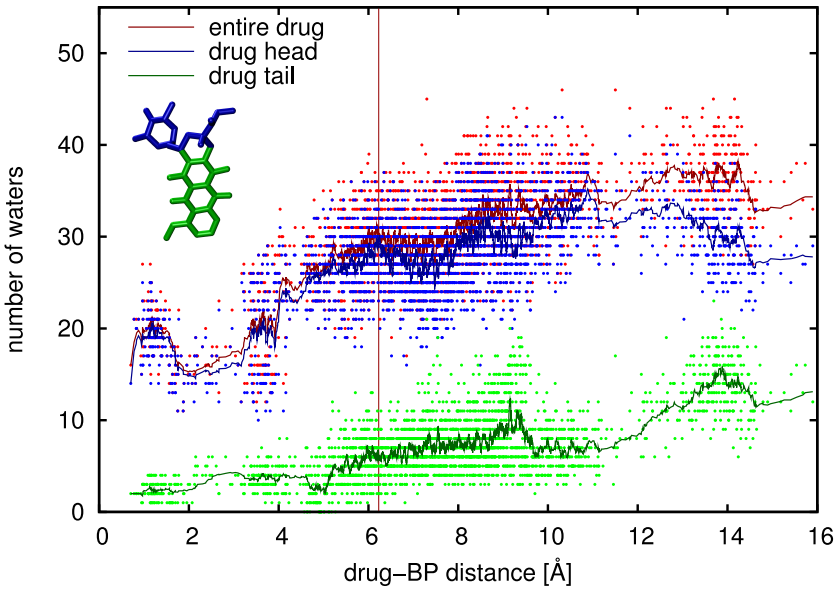

Figure S4: Number of water molecules in the vicinity of the drug, separated by the head and the tail, as a function of the drug-BP distance of all TO simulations including a running average.

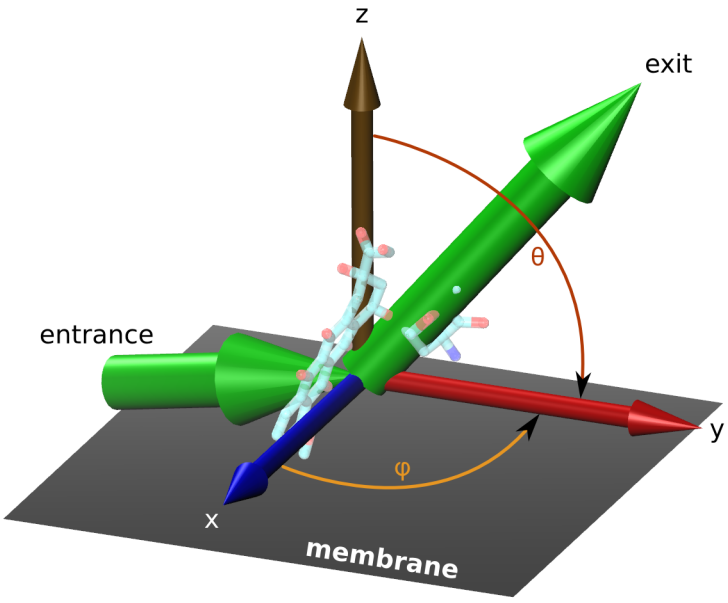

Figure S5: Sketch of the spherical coordinate system and the model path from the entrance through the interior of the pore domain toward the exit. Please note that this is the same path as already indicated in Figure 2. Furthermore, the orientation angles of the drug are shown (ϕ in the x-y plane parallel to the membrane and θ down from the z axis to the major axis of the substrate).

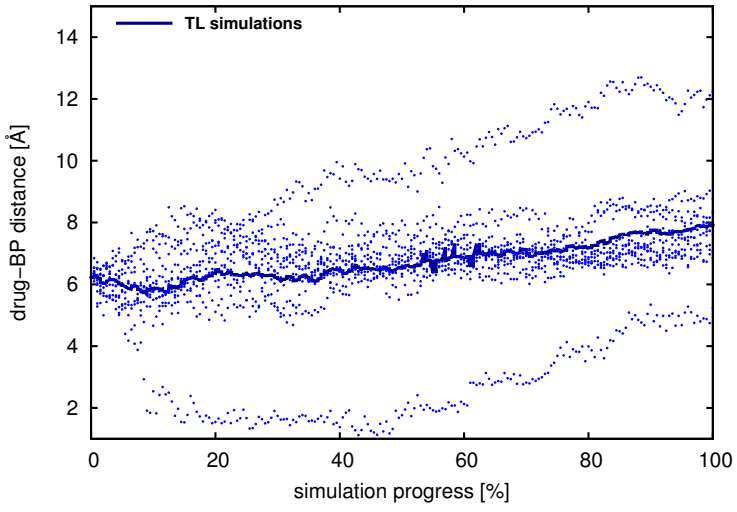

Figure S6: Plot of the drug-BP distance as a function of the relative simulation time. Results from all the simulations are shown as dots, and a running average is represented by the solid line.

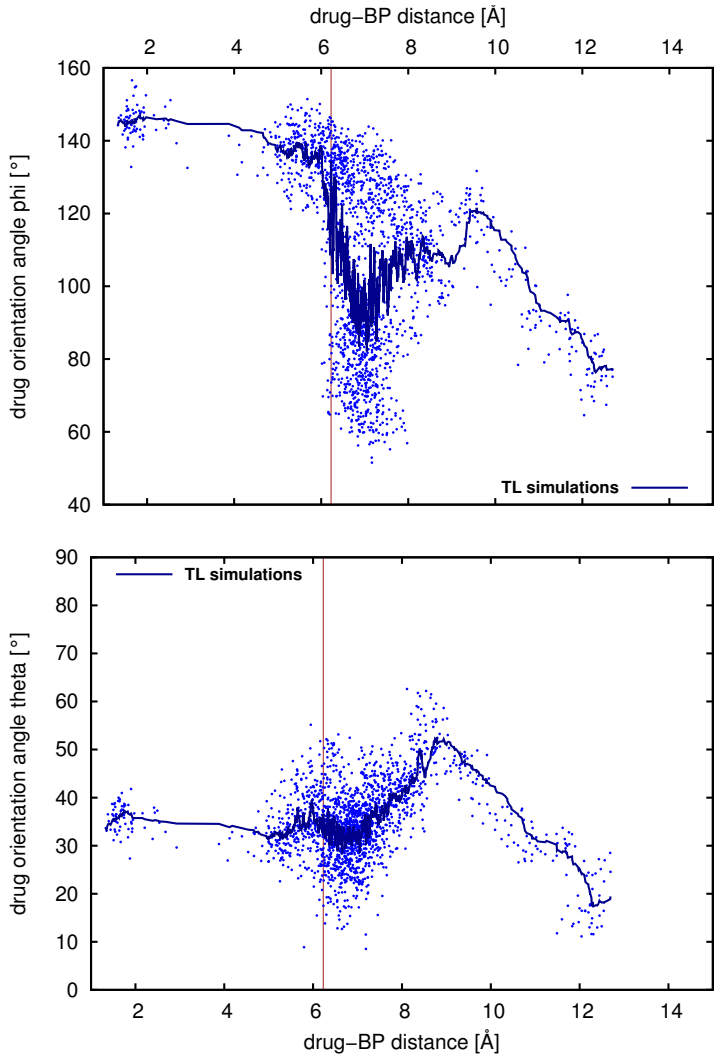

Figure S7: Orientation of the drug in the AcrB pore domain along TL simulations, according to the angles ϕ (upper panel) and θ (lower panel) defined in Figure S5. The vertical line represent the initial state again.

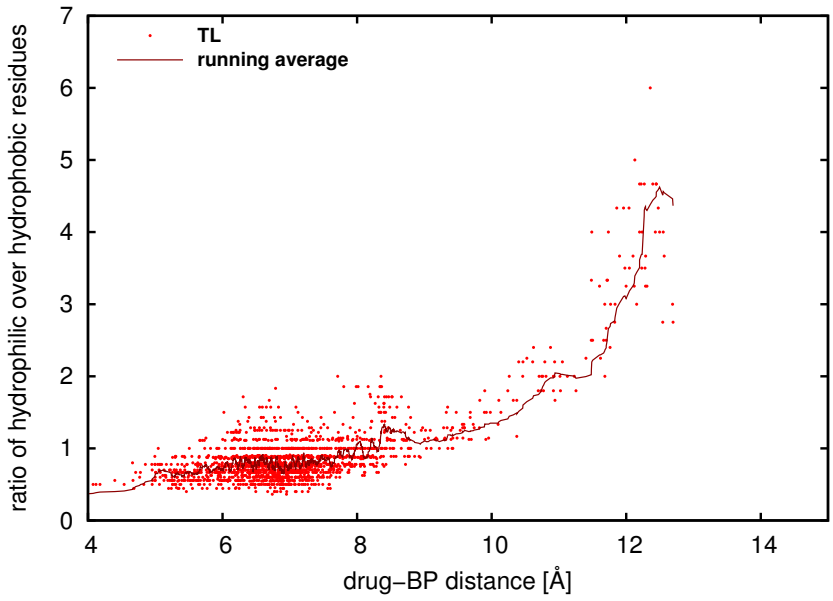

Figure S8: Ratio of hydrophilic over hydrophobic amino acids in the vicinity (3.5 Å) of the substrate as a function of the drug-BP distance of all TL simulations including a running average.