Molecular Dynamics simulations on a bacterial homolog of the human glutamate transporters

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Human glutamate/aspartate transporters cotransport three Na^+ and one H^+ ions with the substrate, and countertransport one K^+ ion (Zerangue & Kavanaugh, 1996). The archeal homolog Glt_{Ph}, which has a 36% sequence identity with the human transporters, transports aspartate and three sodium ions across the lipid membrane. The binding sites for the substrate and two Na^+ ions have been observed in the crystal structure of Glt_{Ph} (Boudker *et al.*, 2007), while the binding site for the third Na^+ ion (Na3) has been proposed from computational studies and confirmed by experiments (Bastug *et al.*, 2012). Here we perform detailed free energy simulations of Glt_{Ph} in the extracellular(EC) and intracellular(IC)-facing states, giving a comprehensive characterization of the substrate and sodium binding sites, as well as calculating their binding free energies in various configurations (Heinzelmann, Bastug & Kuyucak, 2011, 2012). Our results from the EC-facing state show unequivocally that the substrate binds after the binding of two Na^+ ions, with a strong water-mediated coupling between the second sodium ion to bind (Na1) and the substrate. In the IC-facing state, even though the gating mechanism is different, the unbinding of the ligands happens in a symmetrical manner, with the ligands being released in the reverse order of their binding. The Na3 ion is the first ligand to bind and the last to unbind, and we show by free energy calculations and experiments that the latter is the rate-limiting step in the transport mechanism.

Bastug T, Heinzelmann G, Kuyucak S, Salim M, Vandenberg RJ, Ryan RM. (2012) *PLoS ONE* **7**: e33058.
Boudker O, Ryan RM, Yernool D, Shimamoto K, Gouaux E. (2007) *Nature* **445**, 387-393.
Heinzelmann G, Bastug T, Kuyucak S. (2011) *Biophysical Journal* **101**, 2380-8.
Heinzelmann G, Bastug T, Kuyucak S. (2012) *Biophysical Journal* submitted for publication.
Zerangue N, Kavanaugh MP. (1996) *Nature* **383**, 634-637.