

Free energy simulations of Asp/Glu transporter GltPh

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Glutamate is the dominant excitatory neurotransmitter in the brain. Its extracellular concentration is kept at the nanomolar level by glutamate transporters – membrane proteins that continuously pump glutamate back to the neurons using the existing ionic gradients. Because small changes in glutamate concentration have major effects in signaling in the brain, glutamate transporters are key targets for treatment of various neurological conditions. Here we use the recently determined bacterial transporter structure GltPh in MD simulations to delineate the basic steps involved in glutamate transport and provide a structural basis for the transport mechanism. We have confirmed the binding sites Na1, Na2 and Asp, suggested by the experimental structure, and found a third Na ion binding site (called Na3), which is critical for the functioning of the transporter. This also strengthens the case for constructing homology models for human glutamate transporters (EAAT1-5) from the GltPh structure. Our proposed binding site for Na3 is completely consistent with the structural data, which is not the case for other proposed sites.

We have calculated the binding free energies for Na ions and Asp in various configurations and thus determined the order of binding as Na3, Na1, Asp, Na2 (following the notation used in the crystal structure). To understand the selectivity of GltPh for Asp, we have performed free energy perturbation calculations for the transformation Asp to Glu and found a selectivity free energy barrier of 4-5 kcal/mol consistent with the experimental observations. This is basically caused by steric interactions - the larger sidechain of Glu does not quite fit in the binding site. Once we understand the operation of the bacterial transporter, we will create a homology model for the mammalian glutamate transporter EAAT1 and investigate similarities and differences in the transport mechanism with the bacterial one, following steps similar to above.