Computational characterization of proton coupling in Xyle

Sugar transporters have the fundamental role to take up energy into cells in the form of glucose and other hexoses; they belong to the major facilitator superfamily (MFS) and follow a rocker-switch alternating access model of transport. XylE, an *E. coli* homolog, has been used as a model to study functional characteristics of GLUTs. Yet, in contrast to most of the GLUTs, XylE is a proton-coupled sugar transporter. Previous studies have suggested that substitution of one aspartate residue (D27) with an asparagine might be responsible for the difference in coupling. However, the presence of the same aspartate in GLUT2, despite it not being coupled to proton uptake, suggests that the coupling could involve more than simply this residue. Intriguingly, this aspartate belongs to a network of three protonatable residues, one aspartate, one glutamate and one arginine. We speculate that the proton coupling is achieved through an interplay of these residues which could all participate in the proton coupling process.
Our unbiased MD simulations suggest that the stability of different states along the translocation pathway is controlled by the protonation state of this central triad.
Here, we use constant pH simulations in order to characterize the interplay between different residues, allowing for their protonation state to change dynamically.
Unexpectedly, the most stable protonation state differs from the one suggested in resolved structures; this suggests that we do not yet grasp the tight coupling between protonation events and protein dynamics.
We then use enhanced sampling techniques to characterize these protein dynamics in different conformational states.
Finally, combining the two approaches, we strive to study the interplay between the state of all protonatable residues and progression along the transport cycle.