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Title: A biophysical study to investigate oligomer orientations and interface of the human dopamine transporter

The human dopamine transporter (hDAT) belongs to the solute carrier 6 (SLC6) family of transporters and is mainly expressed in the presynaptic specialization of dopaminergic neurons. Its function is to take up the neurotransmitter dopamine after its release into the synaptic cleft, by utilizing existing sodium and chloride concentration gradients over the plasma membrane. Disturbance in its function can lead to Parkinson's disease, bipolar disorder and attention deficit hyperactivity disorder (ADHD). hDAT and other monoamine transporters form stable oligomers at the membrane. Many functional studies have reported that hDAT functions as an oligomer, and some of the interface residues have been proposed. Similar observations have been made in other monoamine transporters. Moreover, the availability of crystal structures of LeuT and dDAT has increased understanding of these transporters. Nevertheless, the oligomeric arrangement of hDAT and especially the residues forming the interface therein are still not well understood. To connect the functional studies with structural details, a reliable *in silico* method is needed due to the complex nature of oligomer formation in the membrane. Hence, to study the oligomerization of hDAT *in silico*, we have utilized a method called DAFT (**D**ocking **A**ssay **F**or **T**ransmembrane components). The DAFT workflow converts a fine-grained hDAT model to a coarse-grained model. A system with two hDAT monomers embedded in a membrane was created in order to provide a simple model of how two monomers interact in the membrane. The monomers were in random orientations seperated by a defined distance, and thus the two monomers do not interact with each other. In total, 512 systems were generated and each was run for 2 µs. These were unbiased simulations with different starting point. Thus, in total >1ms of simulation time was attained. Analysis of the ensembles obtained revealed that hDAT oligomerizes in both symmetric and asymmetric ways with four configurations each. Altogether, eight different predicted orientations are achieved. Additionally, hDAT was able to omit the bundle domain from the oligomer formation. This was supported by potential of mean force (PMF) calculations indicating a flat energy landscape, whereas scaffold domain mediated oligomers indicated a deep energy minimum, suggesting that hDAT has an intrinsic ability to function as an oligomer. The interface mainly involves the helices from the scaffold domain sparing the bundle domain, which is vital for the function of the transporter.