Kinetics of Na⁺ release from the functional Na2 site of the dopamine transporter regulated by the N-terminus

George Khelashvili*, Asghar Razavi and Harel Weinstein

Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY, United States of America

The dopamine transporter (DAT) belongs to the neurotransmitter/sodium symporter (NSS) family of proteins that are responsible for reuptake of neurotransmitters from the synaptic cleft, thus helping terminate a neuronal signal and enabling subsequent neurotransmitter release from the presynaptic nerve. The release of a sodium ion from one of the functional sites identified crystallographically, the Na2 site, has been identified as important mechanistic step in the transport cycle, which prepares NSS for substrate translocation by stabilizing an inward-open conformation. Here, we used extensive molecular dynamics simulations combined with Markov state models to explore the mechanism of Na⁺ release from the Na2 site of the human dopamine transporter (hDAT). Our results show that the initiation of the release process is triggered by hydration of the Na2 site, concomitant with conformational transition from an outward-facing to inward-facing state. Using the Markov model approach we quantify the kinetics of the release process and identify most probable Na⁺ release pathways, revealing the importance of various modes of interaction of the N-terminus of hDAT in controlling these pathways. Furthermore, an intermediate state is discovered in the release pathway of Na⁺ and its kinetics are determined.

*Submitting author e-mail: gek2009@med.cornell.edu