

# Time-resolved Cryo-EM of Glt<sub>TK</sub> - a Glutamate/Aspartate Transporter

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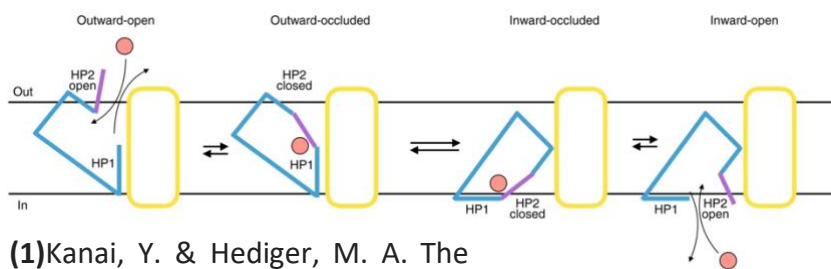
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Glutamate/aspartate transporters are cation-coupled secondary active membrane transporters that clear the neurotransmitter L-glutamate and aspartate from the synaptic cleft.<sup>1</sup> One such transporter, Glt<sub>TK</sub>, presents as a homotrimer, with each protomer functioning independently by an elevator-type mechanism, in which a mobile transport domain alternates between inward- and outward-oriented states. Due to the inherent differences in neuronal concentrations of the amino acid substrates, Glt<sub>TK</sub> is a dynamic protein constantly in motion.

The changeable nature of Glt<sub>TK</sub> is exhibited by the five disparate structures solved by our group using cryo-EM.<sup>2</sup> Each unique state represents a timepoint in the overall transportation process which appears to involve numerous areas and channels on and throughout the transporter protein. Despite these single-particle “snapshots”, our understanding of how Glt<sub>TK</sub> functions has remained incomplete due to a lack of direct observations owing to the fast motions of this transporter. Accordingly, this work complements and builds upon the previous microscopy and crystallographic “snapshots” of Glt<sub>TK</sub> by constructing a molecular movie of transportation using millisecond time-resolved cryo-EM. By controlled freeze/thaw cycles and UV irradiation, we can observe the entire Glt<sub>TK</sub>-substrate transportation event in a stop-start manner at millisecond resolution. Observing the function of Glt<sub>TK</sub> at this resolution allows for the design of site-specific drugs able to target binding sites previously unobserved in static structures.



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- (2) Arkhipova, V., Guskov, A. & Slotboom, D.J. Structural ensemble of a glutamate transporter homologue in lipid nanodisc environment. *Nat Commun* **11**, 998 (2020).

<https://doi.org/10.1038/s41467-020-14834-8>

