Different location same story: Identification of a common binding site for membrane-anchored ancillary proteins in the SLC6 family of neural and epithelial transporters

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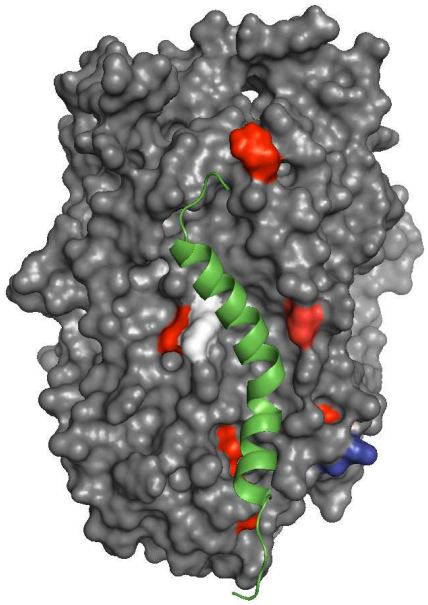
Syntaxins are single-pass membrane-anchored proteins, which in their classic role form part of SNARE complex mediating vesicular fusion events. The first syntaxin to be characterized in this role was syntaxin 1A – a T-SNARE facilitating neurotransmitter-containing vesicular fusion at neuronal synapses. Another well-known role of syntaxin 1A is its functional interaction with a wide range of membrane transporters and channels, including the GABA, dopamine, and norepinephrine transporters of the Neurotransmitter Sodium Symporter (NSS)/SLC6 family. Close homologues to these NSS neurotransmitter re-uptake transporters are two kidney epithelial transporters of the SLC6 family B^0AT1 and B^0AT3, also characterized by their association with collectrin, another single-pass membrane protein which act as a molecular chaperone to the transporters. Collectrin has further been implicated in renal aminoacidurias, hypertension, insulin exocytosis and β-cell proliferation in the pancreas.

We show that sytaxin 1A and the kidney specific syntaxin 3 also interact with B^0AT1, and collectrin with the GABA transporter. Consistent with their role in regulating neurotransmitter transporters, syntaxins inhibit B^0AT1 trafficking to the plasma membrane, whilst the effect of collectrin is to stimulate trafficking. We also investigated the possibility that the binding of these unrelated ancillary proteins represents a common competitive binding site conserved across SLC6 transporters. In addition, and unexpectedly, we also discovered collectrin is essential for the catalytic activation and functional complex formation of both B^0AT1 and B^0AT3. Sequence alignment, structural considerations and mutational analysis suggest that amino acid residues clustering around a cavity formed by trans-membrane helices 5 and 7 are vital for collectrin-mediated membrane stabilization and functional activation. These findings demonstrate that B^0AT1 is a heteromeric transporter forming a functional complex with collectrin and possibly competing with syntaxin proteins at a common SLC6 binding site.



Hypothesized interaction site between collectrin's TM domain (Met-136 –Arg-171) and the groove formed by helices 5 and 7 in B^0-like transporters. The transporter is viewed parallel to the membrane with the POPC membrane used during MD simulation removed to see the proteins clearly. B^0AT3 is visualized in gray with collectrin Met-136 to Arg-171 in dark green. Important functional residues for collectrin interaction in the transporter are coloured red (decreased transport) or blue (augmented transport), white residues showed no transport activity change. The model is based on the end point of a 10-ns MD simulation.