Towards the transport mechanism of the human serotonin transporter: computational investigations of an outward-occluded state

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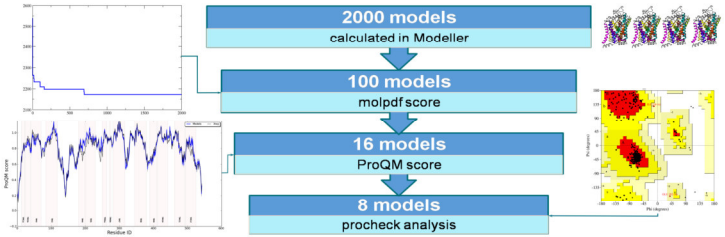
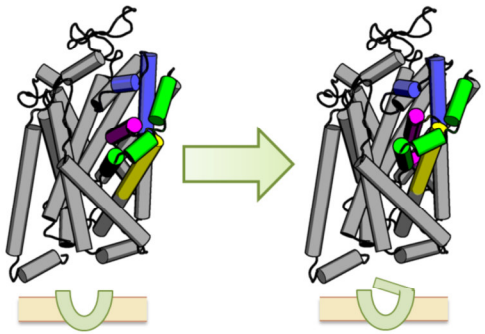
The human serotonin transporter hSERT

- ★ regulates serotonergic transmission
- ★ important drug target interacting with various compound classes
- ★ the mechanic characteristics of transporter substrates and inhibitors remain often elusive
- ★ the role of transporter transition to different conformations in this context needs to be established

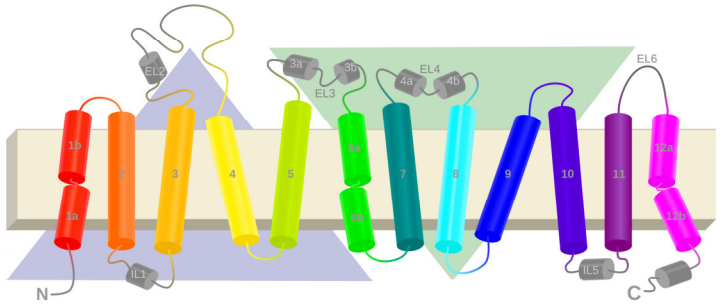
Homology modeling of an outward-occluded state



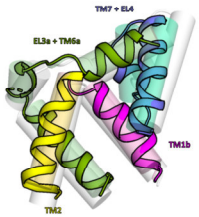
- ★ X-ray structure in outward-open (ce) state provided starting point for molecular modeling and MD simualtions
- ★ homology model of hSERT based on a combination of two templates in a new outward-occluded (ceo) state (5HT)
- ★ thorough validation and careful refinement process
- ★ induced-fit docking study with serotonin and subsequent common scaffold clustering
- ★ in parallel, same docking and clustering approach with the X-ray structure (ce)
- ★ microsecond long MD simulations with both ce and ceo systems



hSERT ce vs. hSERT ceo state

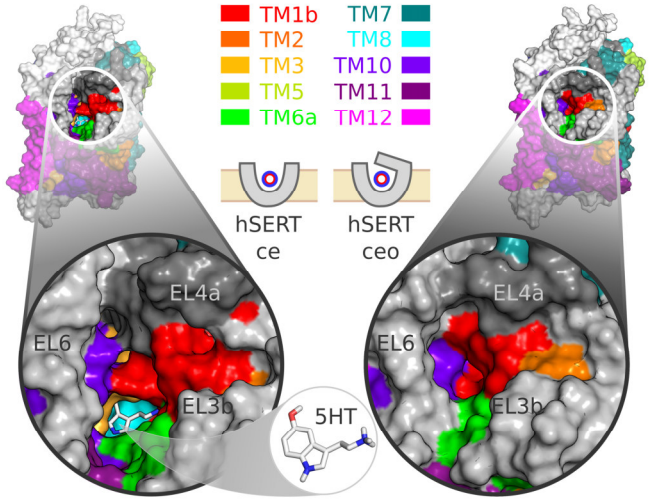


The transition from ce to ceo state in hSERT requires changes in TM1b, TM2 (upper part), EL3b, TM6a, TM7 (upper part) and EL4a which were taken from the bacterial homolog transporter LeuT 3F48 (ceo), the remaining parts came from hSERT 5I71 (ce)



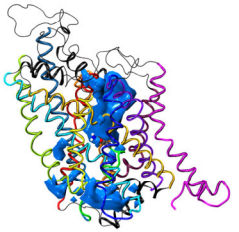
For the modeling process, TM1b, TM2 (upper part), EL3b, TM6a, TM7 (upper part) and EL4a of hSERT 5I71 were fitted to the corresponding parts in LeuT 3F48 and then used as a template for the homology model of hSERT ceo.

The central binding site (S1) for serotonin in hSERT is accessible from the extracellular side in ce, but not in ceo state.

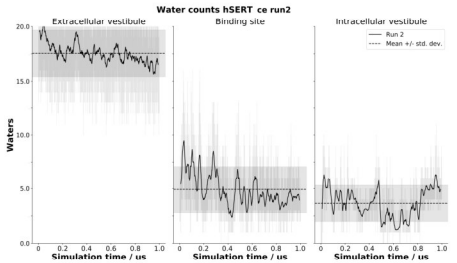


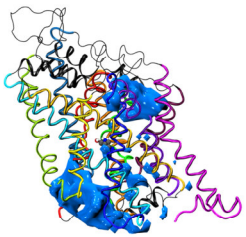
Insights from MD simulations

Water occupancy maps and water molecule counts over time show solvation of the extracellular vestibule in ce state and of the intracellular vestibule in ceo state.

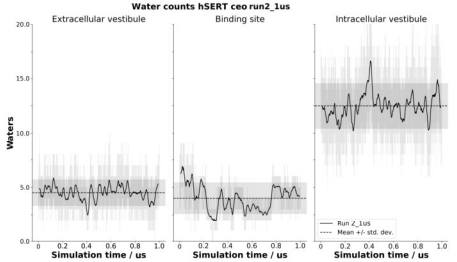


hSERT ce run2 (1us)

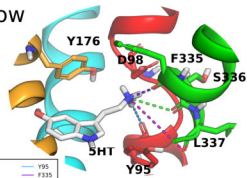


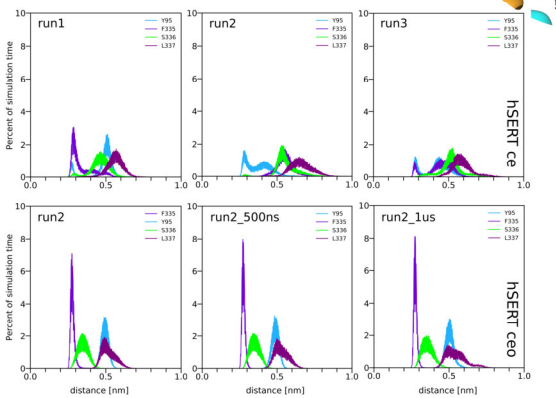


hSERT ceo run2_1us (1us)



Distance analyses in the S1 binding site show that the cationic nitrogen of serotonin is sampled in vicinity to Y95, F335, S336 and L337 - but only in the ceo simulations the distances stay short over time.





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