Fact?: in transporters as LeuT, \_\_\_\_\_\_\_ the binding of a second substrate in S2 triggers the mechanism of the release of the ions and the substrate inside the cell by hydration or dehydration of the binding sites (different in GltPh). TCAs bind in S2 too but don’t trigger the release, so they actually block the binding of the second substrate and inhibit transport.

Hypothesis?: in other transporters as \_\_\_\_\_\_, the interaction of a tryptophan with the residues in S2 might trigger the release, with a similar/unknown/different mechanism?

* Check if openings/closings of EV caused by that tryptophan imply a closing/opening in the IV by counting the number of molecules in each vestibule and measuring the distance among the residues in S2 and the tryptophan.

If the answer is yes…

Hypothesis: efflux works like transport but backwards: N-terminus changes unstabilize the Na atom and triggers a reduction of volume of the IV. That reduction in IV makes that tryptophan to move outwards and then EV gets bigger and opens. If there is a DA in an “S3” place in the IV, it might make all the way to the EC part.

* Check how the mechanism opening of IV – movement of tryptophan works by applying NbIT? Use LeuT first as control.
* Find whether S3 exist or not by doing MD simulations with more DA in the intracellular part of the protein.