In silico description of OCTN1 recognition mechanism and the role of sodium in substrate binding

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Role of OCTN1 in physiology and pathology

The Organic Cation Transporter Novel 1 (OCTN1), also known as ergothioneine transporter (ETT), facilitates the transport of organic cations, zwitterions, with selectivity for positively charged solutes.[1] Ergothioneine, an antioxidant compound, and acetylcholine (Ach) are among its substrates.[2]

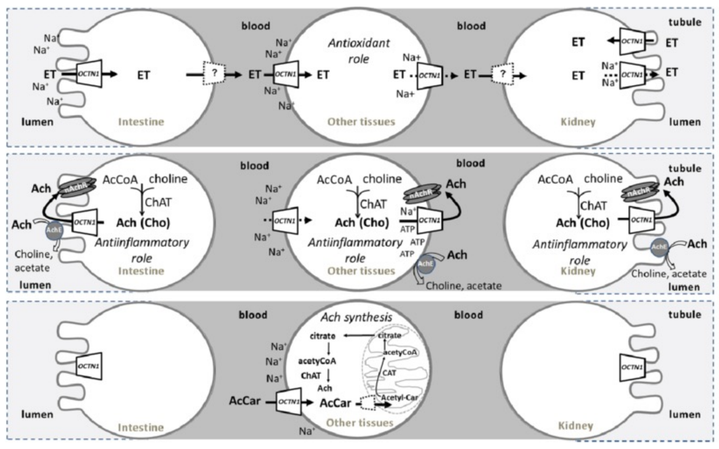
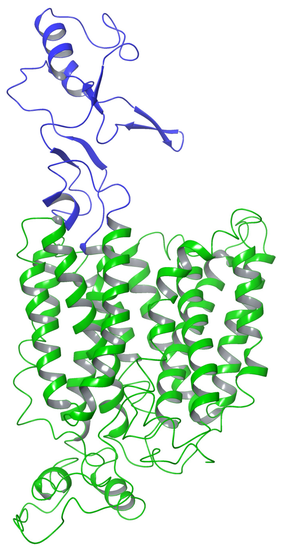


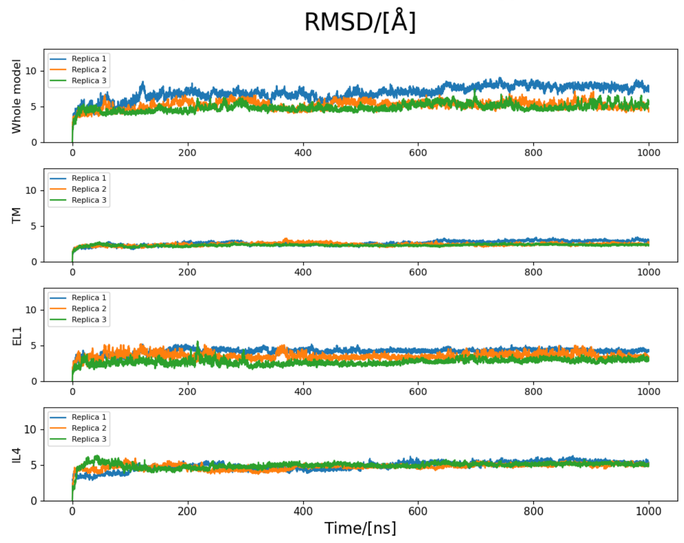
Figure from Pochini *et al.* [1]

Variants of the OCTN1 gene are linked to a heightened risk of inflammatory diseases, such as ulcerative colitis and Crohn's disease. Research demonstrated a significant correlation between OCTN1 expression in cancer cells and patient survival rates, highlighting OCTN1's crucial role in transporting various exogenous compounds, including some anti-cancer drugs.[3] Gaining a deeper understanding of the structure and transport mechanisms of OCTN1 could drive the optimization of such compounds

3D modelling of OCTN1

The 3D structure of OCTN1 has not been experimentally solved, yet. A homologous protein that could be used as a template for comparative modelling was identified in OCT3 (PDB code: 7ZH6), co-crystallized with cortisone and in the outward facing conformation. Because of the low sequence similarity in the first extracellular loop, a chimeric model was generated by grafting the loop generated by AlphaFold2 over the homology model.

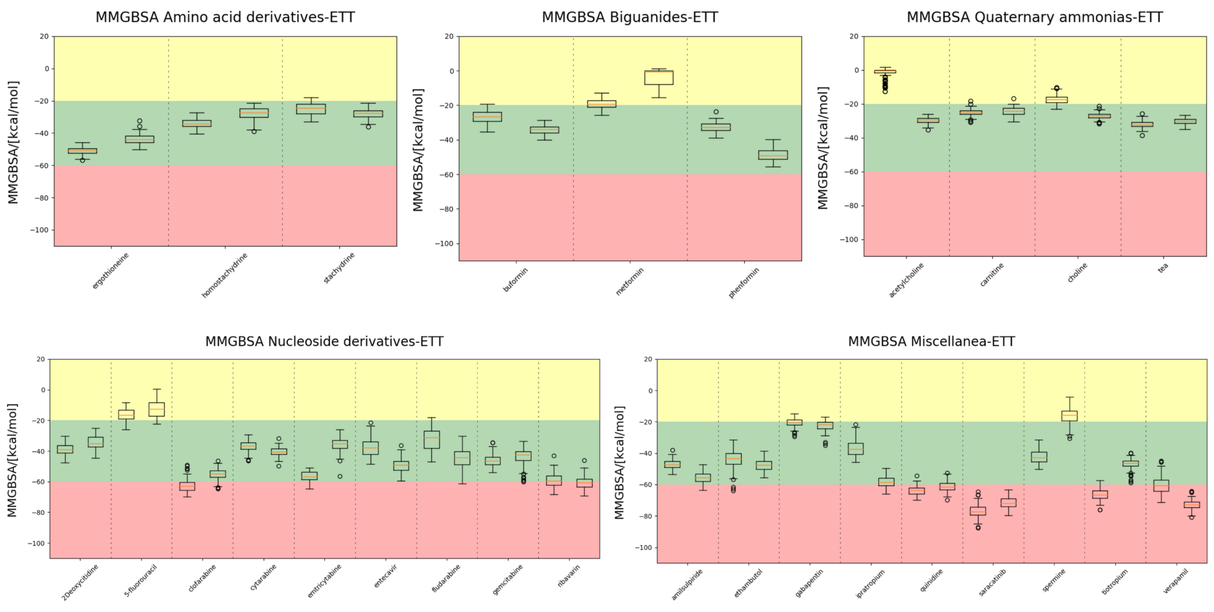




Three replicas of MD simulations were run to assess the stability of the model. While the structure is overall stable, the EL1 and IL4 showcase significant mobility compared to the rest of the protein.

Evaluating the affinity of OCTN1 with substrates and inhibitors

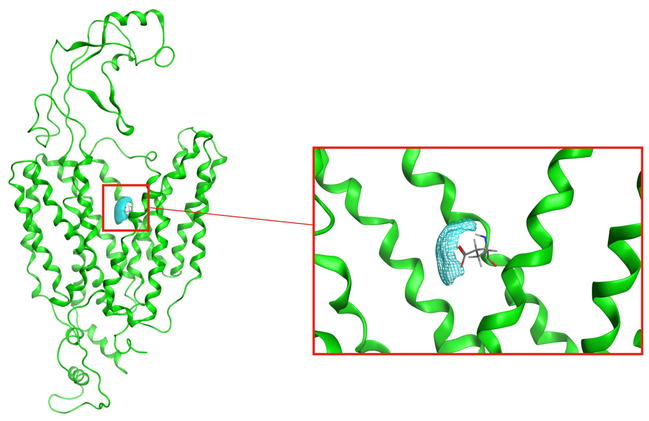
Molecular docking calculations were performed to generate reliable complexes to be used for subsequent MD simulations that could be used to assess the interaction energies during time. MM/GBSA was calculated over two replicas for each complex. The energy values allowed the identification of three groups: weak binders (yellow), good binders (green), and inhibitors (red). These results are consistent with literature data.



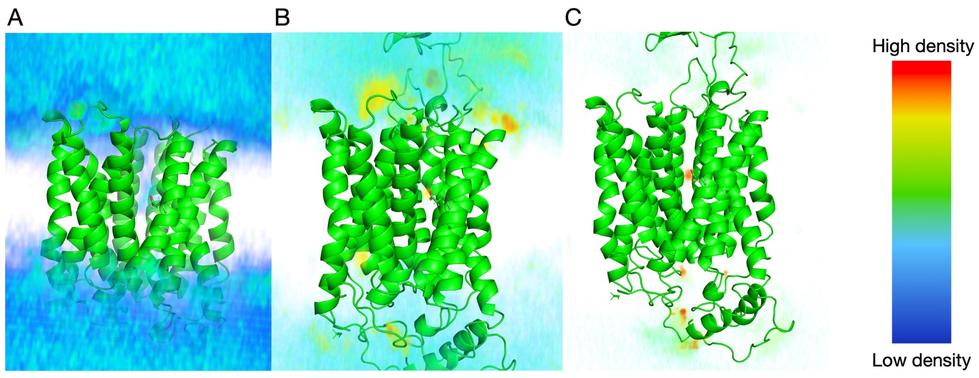
Key residues were identified as interacting with most of the evaluated molecules. Specifically, hydrogen bonds and salt bridge interactions were most frequently generated by Ty211, Glu381, and Arg469

Assessing the role of sodium in OCTN1 activity

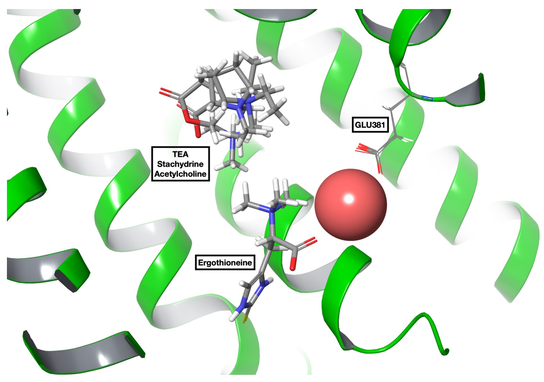
Literature has shown that OCTN1 activity is directly dependent on sodium concentration. However, the exact mechanism is still unknown, and this behavior seems to change depending on the transported molecule. The first step to assess the sodium interactions in the transport funnel was the use of 3D-RISM to locate where the ions are more likely to accumulate. A region was clearly identified around Glu381, the only positively charged residue in the transport funnel.



To corroborate this result, three replicas of MD simulations with increasing NaCl concentrations were run (150mM, 500mM, and 1000mM), and the regions with the highest sodium permanence assessed. These results confirmed the preliminary result obtained with 3D-RISM, highlighting a concentration-dependent behavior.



Molecular docking calculations were performed again considering the sodium ion as part of the receptor. Interestingly, ergothioneine appears to be the only molecule which significantly interacts with the ion, supporting the hypothesis that its transport is the only one dependent on sodium concentration



Conclusions

- In this work, a reliable, chimeric model of OCTN1 was generated and equilibrated.
- Molecular docking and MD simulations of a wide array of compounds allowed the identification of key residues for OCTN1 recognition and an energy-based classification of the class of the compounds.
- The molecular mechanism underlying the role of sodium in OCTN1 activity was explored, identifying Glu381 as a key residue for sodium recognition
- The atomistic description of OCTN1 can become a significant first step for the development and optimization of small molecules that could be used for cancer treatment.

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