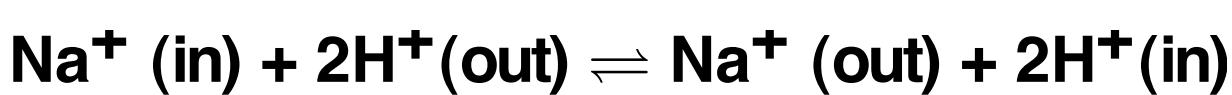


Na+/H+ exchangers

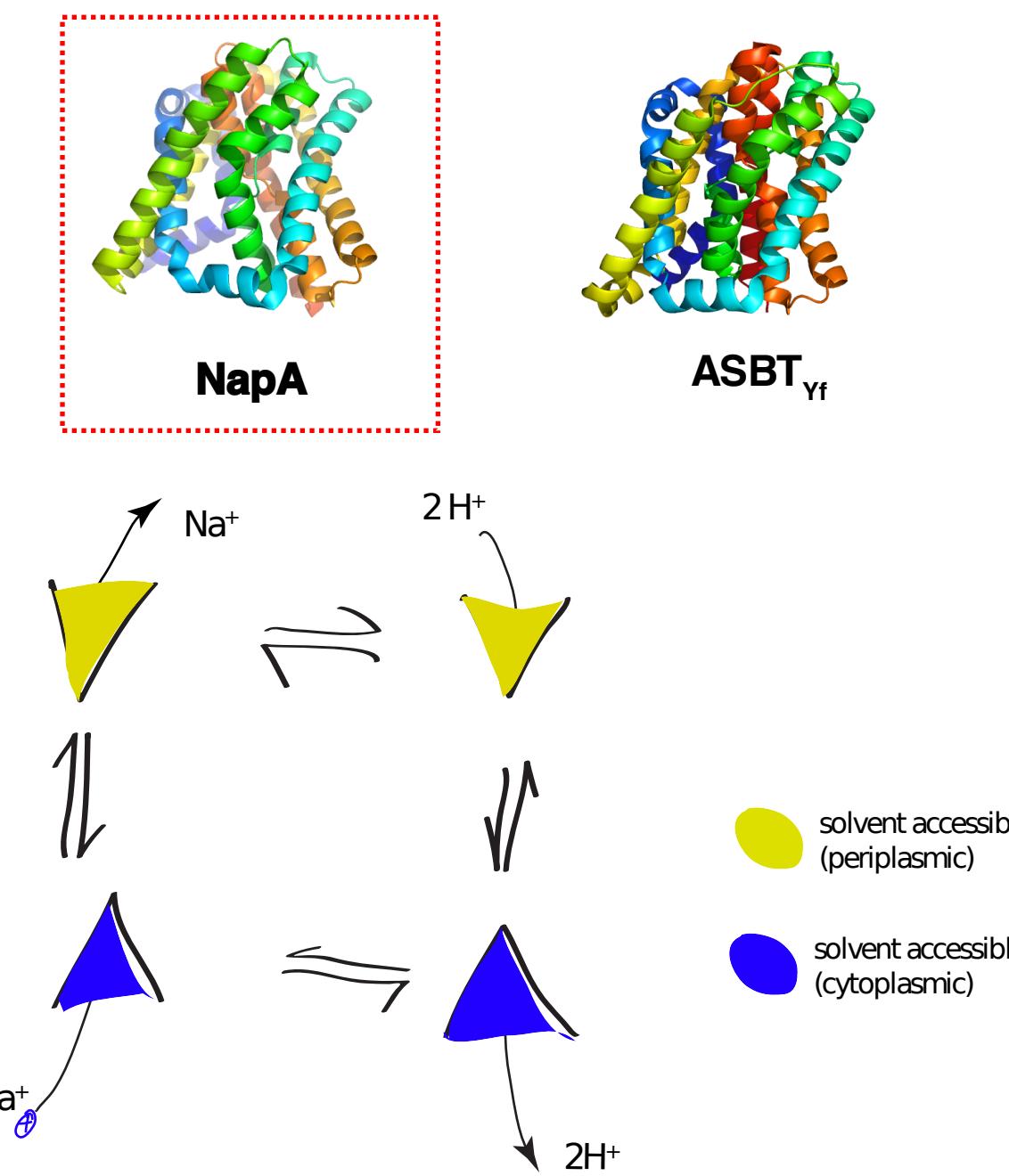
NhaA is a secondary transporter of *E. coli* that uses the proton gradient to extrude sodium ions to facilitate bacterial growth under salt stress.



Transport is electrogenic and fast (1500 ions/s) and pH regulated (inactive pH < 6, fully active at pH ≥ 8).

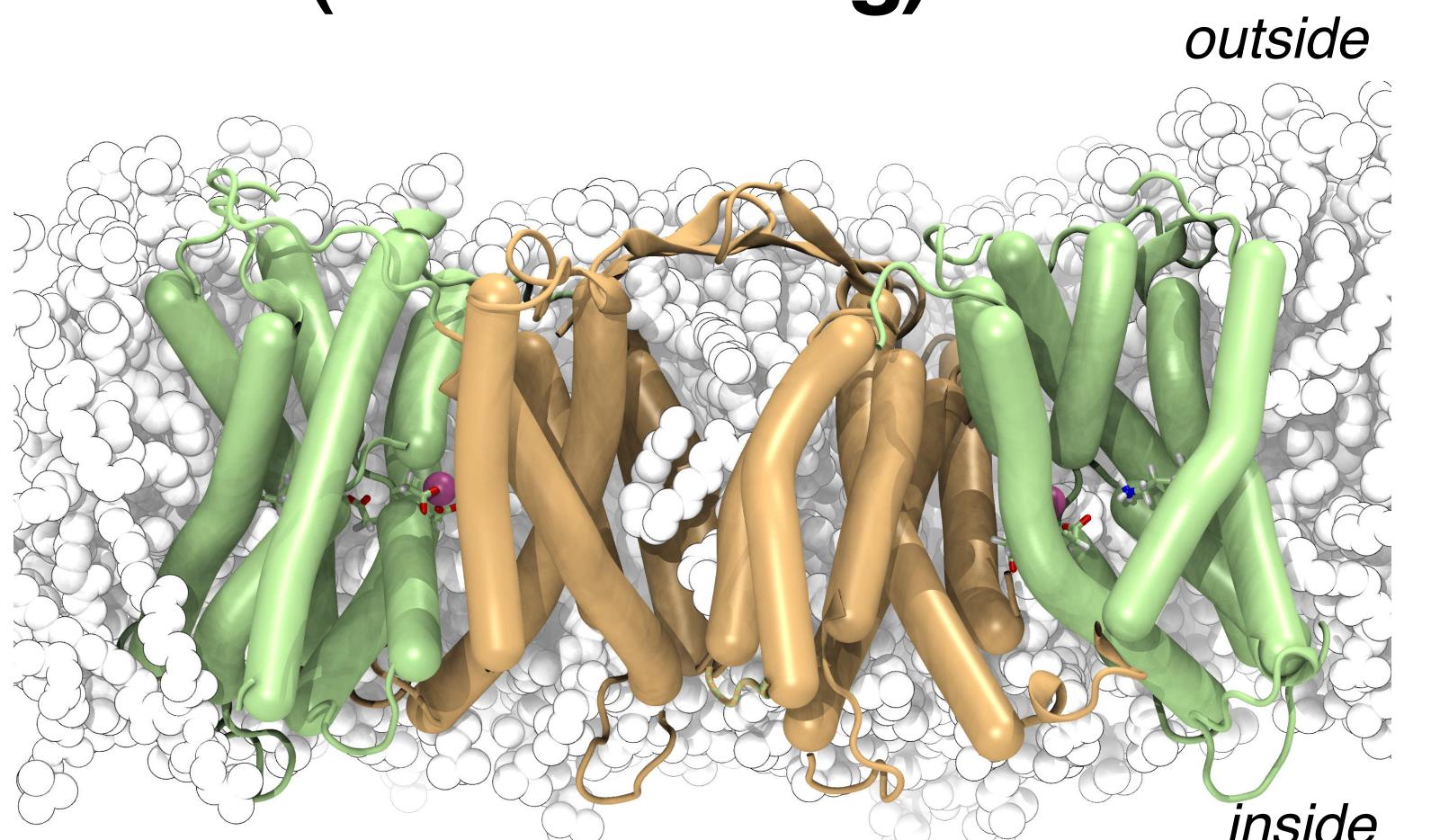
In humans, sodium/proton exchangers (NHE) regulate cell pH by utilizing the Na⁺ gradient to extrude protons in an electroneutral fashion (1 Na⁺ : 1 H⁺).

It is believed to function according to the *alternating access mechanism*. An inward-facing structure of NhaA at low pH is known (Hunte et al, 2005) together with a number of structural homologs. In particular, we solved NapA in an outward facing conformation (Lee et al, 2013), which together with the NhaA structure outlines the conformational changes required to expose a number of conserved charged residues to the periplasm or the cytoplasm. [ASBT_{YV} (bile acid transporter homolog) was also recently solved in inward facing and outward facing states (Zhou et al 2014).]

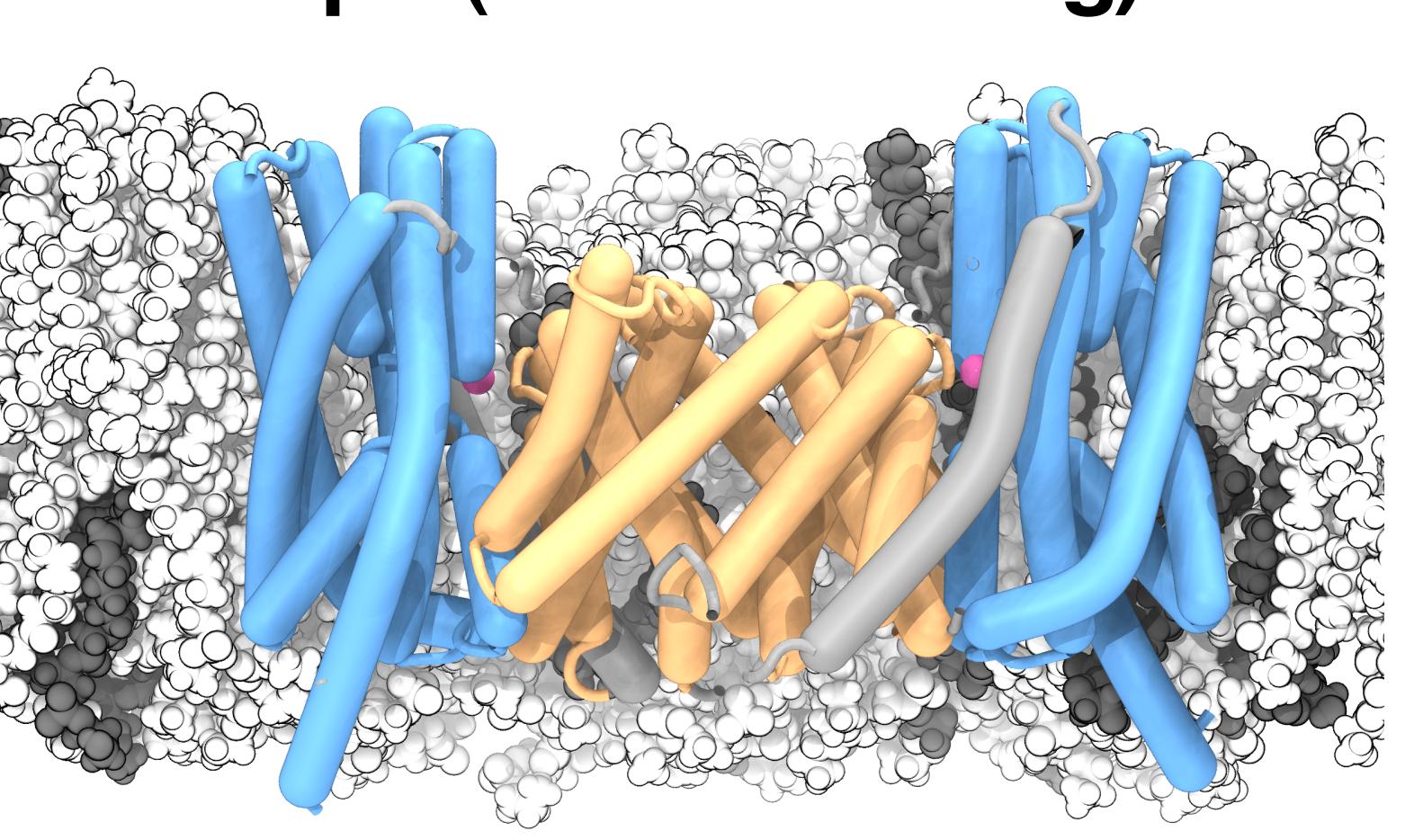


Molecular dynamics simulations of NhaA and NapA

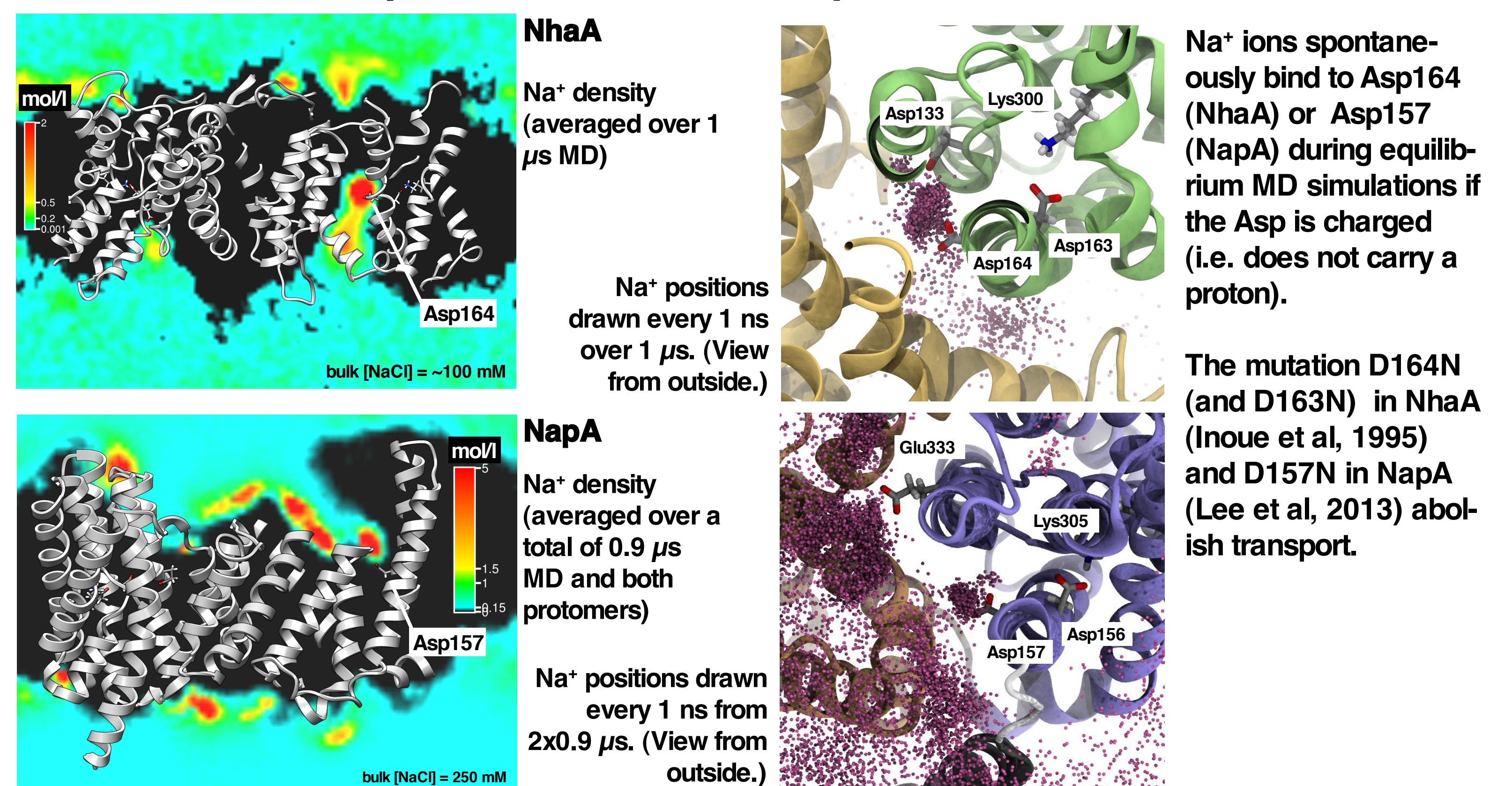
NhaA (inward facing)



NapA (outward facing)



Na⁺ ions bind to equivalent conserved Asp residues



Na⁺ ions spontaneously bind to Asp164 (NhaA) or Asp157 (NapA) during equilibrium MD simulations if the Asp is charged (i.e. does not carry a proton).

The mutation D164N (and D163N) in NhaA (Inoue et al, 1995) and D157N in NapA (Lee et al, 2013) abolish transport.

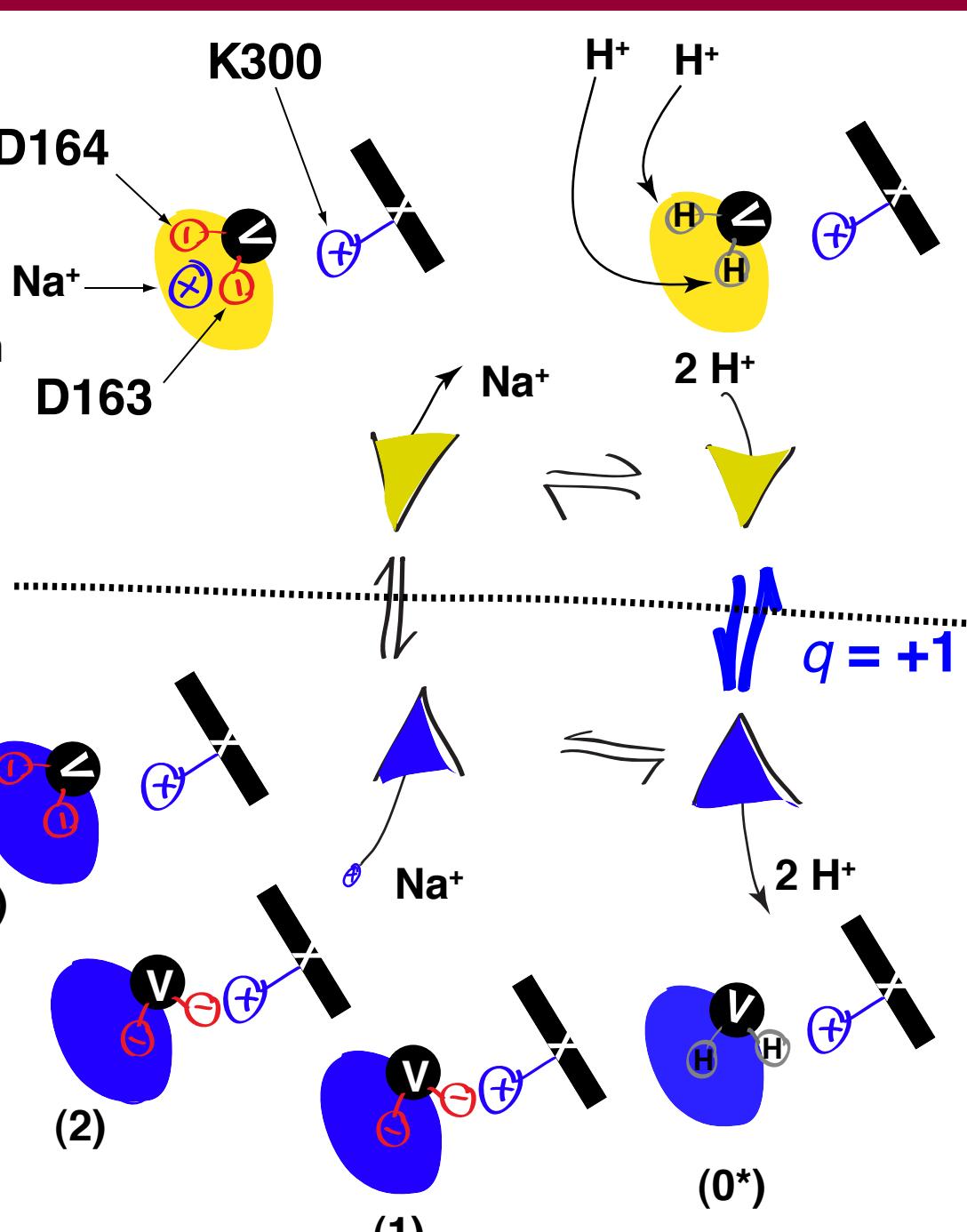
Competitive binding schemes for Na⁺/H⁺ antiport

Aspartates D163 and D164 as proton carriers

Mager et al (2011) have shown that sodium and proton binding is competitive. They propose a model in which D163 and D164 form the binding site.

Based on the MD simulations the observed salt bridge would probably only be transiently occupied.

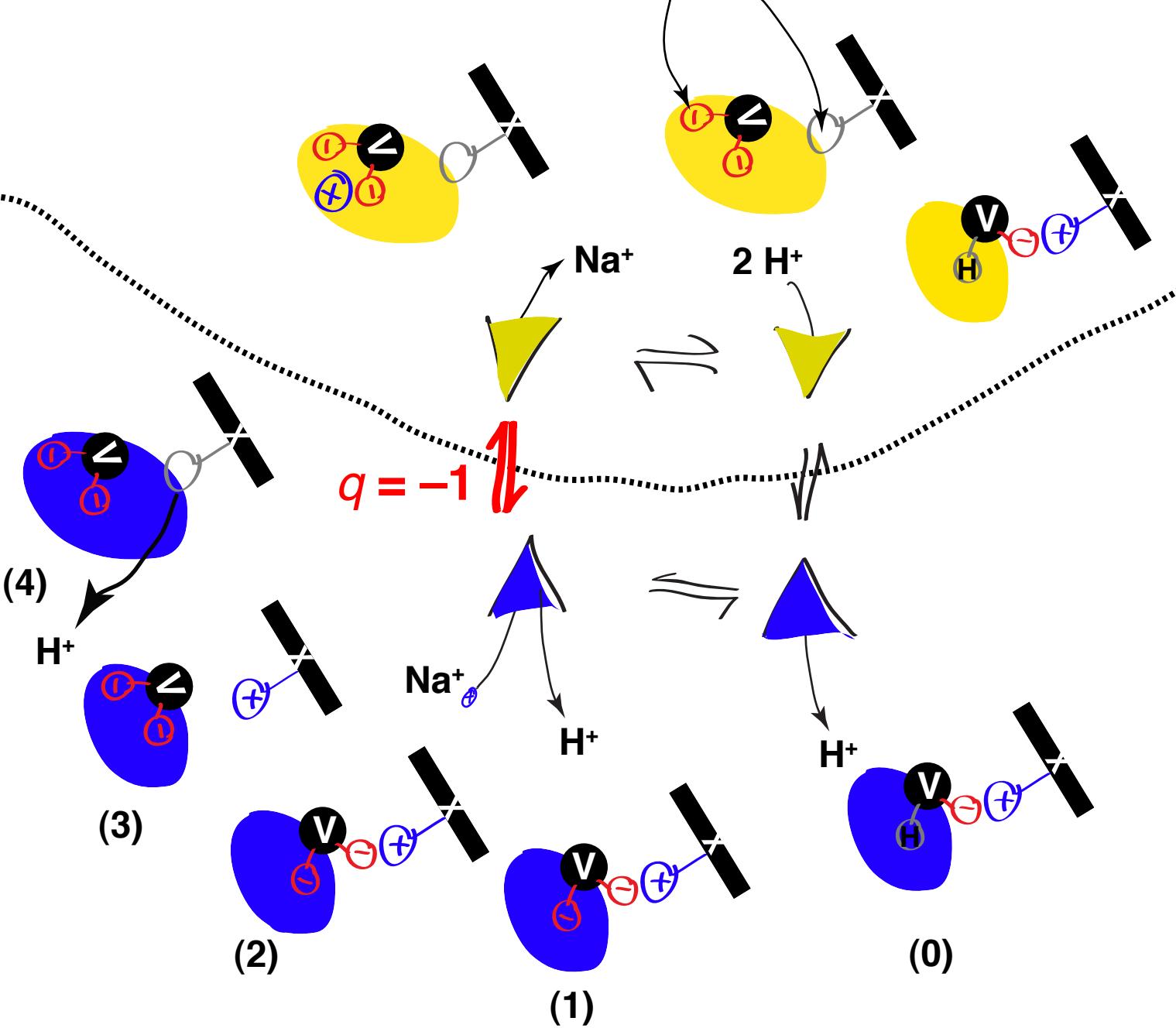
Assuming no other protonation state changes and that only D163, D164, K300 (and Na⁺) switch accessibility, a bound charge displacement of +1 is predicted and the conformational change in the proton translocation step could be membrane voltage dependent.



Aspartate D164 and lysine K300 as proton carriers

The pK_a of lysines in proteins can be shifted from ~5.5 to 10.4 due to their environment. The essential K300 could function as a proton carrier.

A competitive binding scheme with D164 as the second proton carrier accounts for all MD observations in a qualitative manner. The D163-K300 salt bridge is present (especially under low-pH conditions (state (0) could correspond to the crystal structure); presence of Na⁺ weakens the salt bridge [(2), (3)]) and allows the release of the proton from K300, further increasing Na⁺ binding to D163/D164 (4). The scheme predicts a bound charge displacement of -1 and the conformational change in the Na⁺ transport step could be voltage dependent.

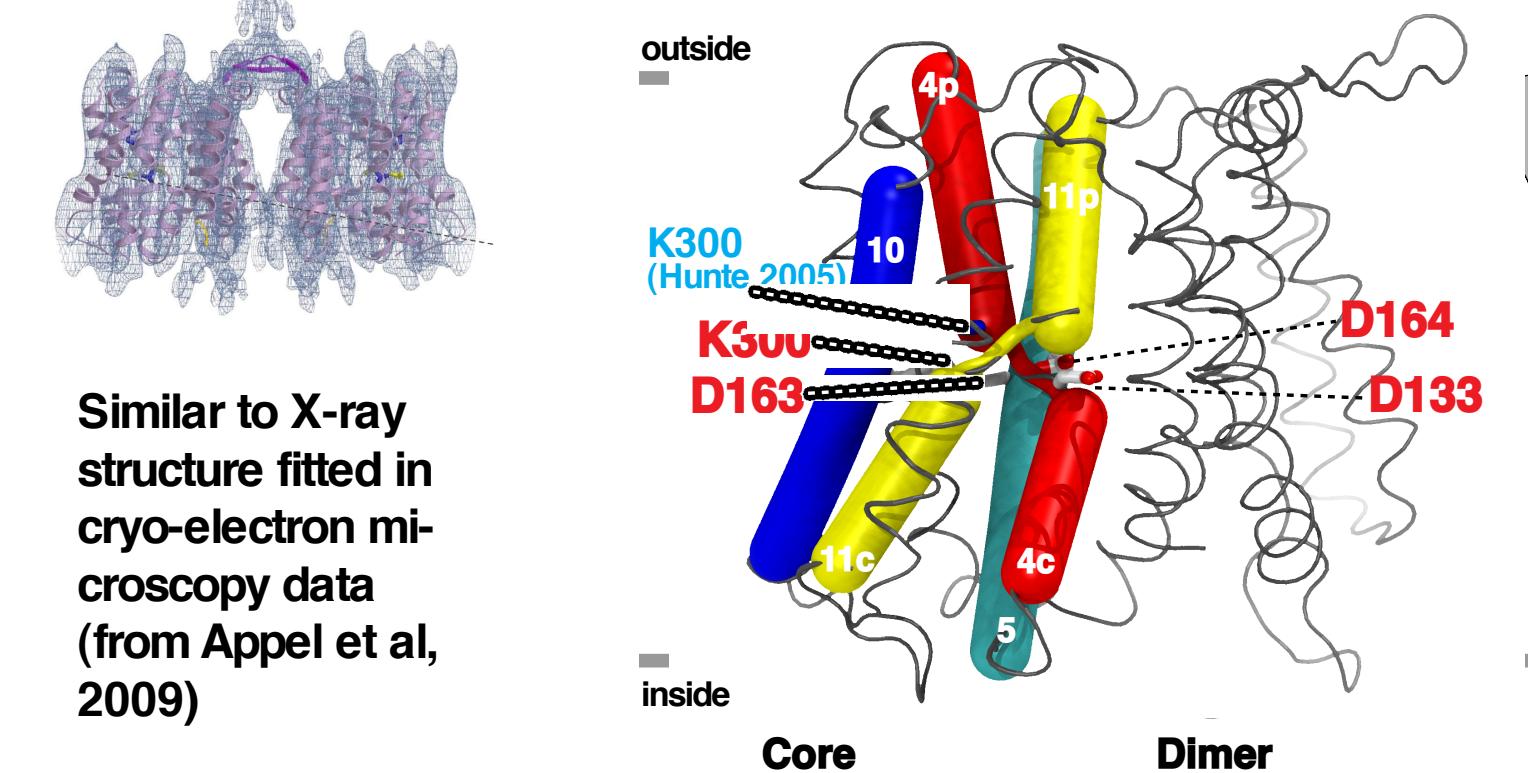


ASBT_{YV} (Na⁺ coupled) shows Na⁺ ion in exact same position as NhaA's K300.

Electroneutral transporters (1H⁺/1Na⁺) replace "Asp163" with Asn and "Lys300" with Arg.

Heuristic pK _a prediction [PROPKA (Li et al 2005)], Na ⁺ not included. MD @ t = 1 μs			
conformation state	X-ray	MD _A	MD _B
(1) yes	(1)	(3)	no
D163	3.9	2.0	5.8
D164	6.9	5.1	7.3
K300	10.3	12.9	8.5

New dimeric crystal structure of NhaA



resolution 3.7 Å (WT),
3.5 Å (SelMet triple mutant), pH 3.8
inward facing

Similar to X-ray structure fitted in cryo-electron microscopy data (from Appel et al, 2009)

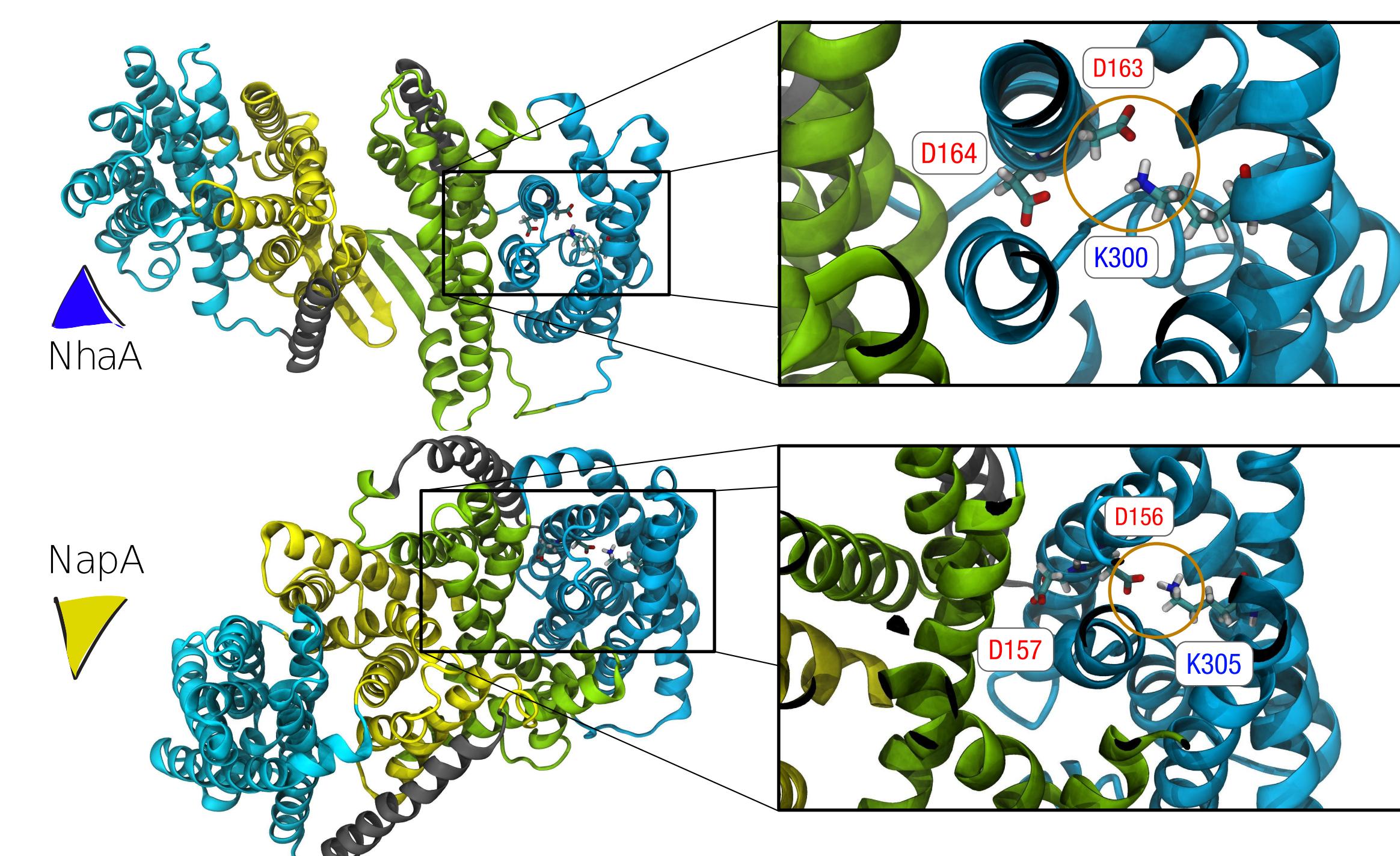
Revised position of K300

helix TM 10 based on Hunte 2005 structure

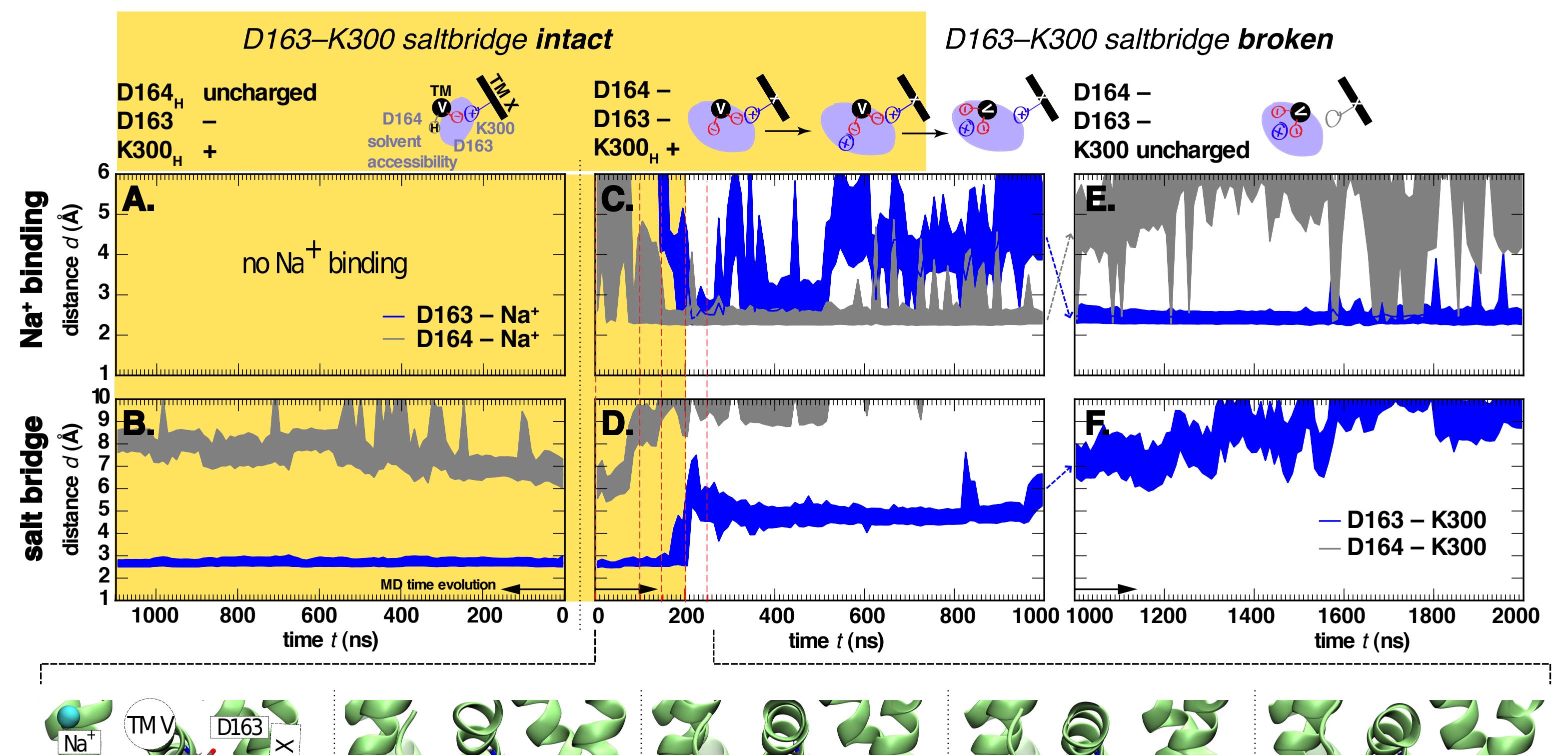
helix TM X rebuilt



Salt bridge D163–K300 (NhaA)/ D156–K305 (NapA)

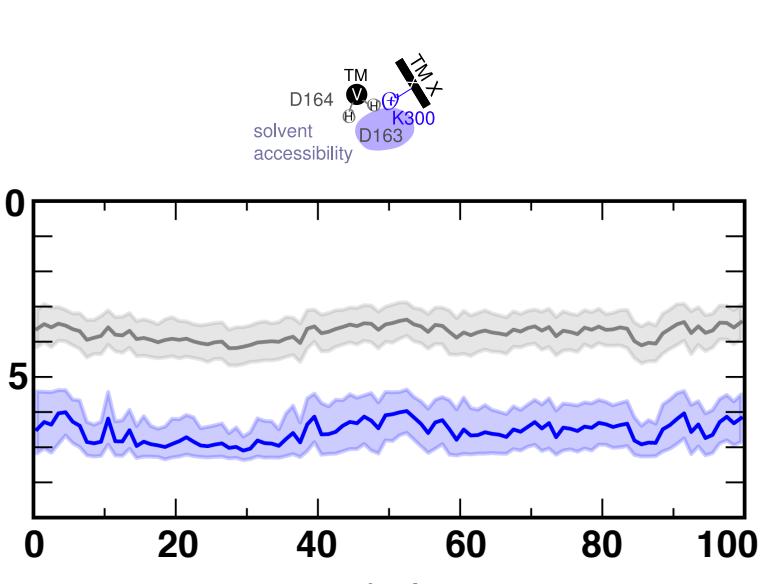


Salt-bridge stability in NhaA may depend on bound Na⁺ ion



Destabilization of the salt bridge by a bound Na⁺ ion was observed in multiple repeat simulations (data not shown). All residues are water accessible, including K300 after ion binding (data not shown).

With both D163 and D164 protonated, the salt bridge also remains intact but shows larger fluctuations:



Summary

K300, at a revised position, forms a salt bridge with D163 in our new dimeric NhaA crystal structure, equivalent to the D156–K305 salt bridge in the NapA structure (3 Å resolution, pH 7.8).

Current models of transport assume that two protons are carried by D163 and D164, respectively. However, D163 and K300 are likely to stabilize each other electrostatically in their charged protonation states due to their salt bridge interaction. Thus, it is unlikely that D163 can carry a proton while being close to a (charged) K300. Furthermore, data from our NapA crystal structure in the outward facing state (Lee et al, 2013) indicates that K300 also switches accessibility. In such a model, the effective charge translocation step ($q=+1$) resides with the proton translocation.

We consider an alternative model whereby protons are carried by D164 and K300 in the intact salt bridge and Na⁺ binds to either or both aspartates. This model associates the sodium translocation step with a net charge displacement of -1. MD simulations indicate how Na⁺ would compete with the salt bridge and weaken it sufficiently as to facilitate the release of the proton from K300.

Acknowledgements & References

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- Funding: Royal Society (DD); BBSRC (CL, ADC); MRC (DD); European Union (EIDICT Project) (OB). XSEDE computing resources (OB, DLD).