## Hot and cold running ion pumps

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Crystal structures of the $Na^+,K^+$-ATPase from both a warm-blooded animal (pig) (Morth *et al.*, 2007) and a cold-blooded animal (shark) (Shinoda *et al.*, 2009) have recently been published. Although the structures of the enzyme from these two species appear very similar, we have discovered major differences in their kinetics,

From investigations of $K^+$ occlusion by the phosphoenzyme intermediate of the $Na^+,K^+$-ATPase and its $K^+$-stimulated dephosphorylation *via* stopped-flow fluorimetry we have found that, whereas both enzymes appear to have similar rate constants of $K^+$-occlusion of 370-380 $s^{-1}$, the two enzymes have very different rate constants of dephosphorylation. For the shark enzyme, dephosphorylation proceeds with a rate constant of only 48 (±2) $s^{-1}$ at 24°C and pH 7.4, whereas for the pig enzyme the rate constant is >365 $s^{-1}$. The dephosphorylation is, thus, the major rate-determining step of the shark enzyme under saturating concentrations of all substrates. For the pig enzyme, on the other hand, the major rate-determining step under the same conditions is the conformational E2-E1 transition of unphosphorylated enzyme and its associated $K^+$ release to the cytoplasm. The differences in rate constant of the dephosphorylation reaction of the two enzymes are paralleled by compensating changes to the rate constant for the E2-E1 transition (Kahlid *et al.*, 2010), which explains why the differences in the enzymes' kinetic behaviour have not previously been identified in steady-state kinetic studies of the enzyme's entire reaction cycle.

In mammals, heat generation by the $Na^+,K^+$-ATPase as a by-product of ion pumping is thought to make approximately a 12% contribution towards the maintenance of body temperature. Therefore, the possibility should be investigated whether under physiological conditions the differences in $Na^+,K^+$-ATPase kinetics, which we have identified between a warm- and a cold-blooded animal, could in part be responsible for the higher body temperature of warm-blooded animals.

Khalid M, Cornelius F, Clarke RJ. (2010) *Biophysical Journal* **98**: 2290.

Morth JP, Pedersen BP, Toustrup-Jensen MS, Sørensen TL, Petersen J, Andersen JP, Vilsen B, Nissen P. (2007) *Nature* **450**: 1043-9.

Shinoda T, Ogawa H, Cornelius F, Toyoshima C (2009) *Nature* **459**: 446-50.