Figure 1

**The pump leak equations and their parametric-analytic solution including KCC2 provide a unified model of ion dynamics and demonstrate the importance of the Na+-K+-ATPase in setting transmembrane ion gradients.** (**A**)In the model, a single compartment is approximated by a cylinder and volume changes are equivalent to changes in the cylindrical height. Potassium (green, K+), sodium (orange, Na+) and chloride (blue, Cl-) are included as permeable ions; impermeable anions (magenta, Az) have an average intracellular charge z. The KCC2 transporter, which by default is switched on, causes efflux of equal parts Cl- and K+, and the sodium-potassium ATPase effluxes 3 Na+ parts for 2 K+ parts moved into the cell. (**B**) Different intracellular starting concentrations of the permeable ions do not affect any of the steady state variables. We show the result for Cl- as a time series of chloride concentration (top panel) and volume (bottom panel). (**C**) The ATPase plays a key role in maintaining steady state ion values and volume: volume explosions (bottom panel) with unstable membrane depolarisations (middle panel) and ion concentration shifts (top panel with colours per ion as in **A**) occur from steady state when the ATPase is switched off between 150 and 350 seconds, but the cell recovers when the ATPase is re-activated. (**D**) The parametric-analytic solution including KCC2 (solid lines) predicts time series runs (dots) for varying ATPase pump rate in all parameters: concentrations of the ions with colours as in **A** (top panel); membrane potential (middle) and volume (bottom). The vertical dashes indicate the state at default pump rate.

Figure 2

**Ionic conductances affect the cellular steady state provided non-passive mechanisms control some of the ionic flux: gCl requires active KCC2 transporters to shift chloride homeostasis.** (**A-C**)The parametric-analytic solution was run for varying ionic conductances: in **A** against the sodium conductance gNa; **B** potassium gK; and **C** chloride gCl. Trends in changes in ECl (blue, top panels), Vm (black, top panels), EK (green, top panels) and volume (bottom panels) were compared to values at the default conductance (vertical dashes). (**D**) gCl is varied again but without any flux through KCC2, and thus gCl does not affect the steady state values.

Figure 3

**Shifts in KCC2 flux / conductance cause persistent shifts in the membrane potential and chloride gradient.** (**A**) A ramp increase in KCC2 conductance, gKCC2 (bottom panel), initially started at the default gKCC2 of 10 nS, which contributes towards the total flux through KCC2, causes lasting shifts in ECl (blue, top panel) and Vm (black, top) with minimal changes to EK (green, top) and volume (middle panel). (**B**) The ATPase rate can cause shifts to the steady state ionic gradients (top panel, colours as for **A**) and volume (bottom panel), but these variables are relatively stable near the default pump rate (vertical dashes). (**C**) gKCC2 can shift the steady state variables as well as the driving force (bottom panel) as indicated for **B**, and these are susceptible to small shifts in gKCC2 near its default value (vertical dashes). (**D**) [Meta-analysis]

Figure 4

**Changes in the concentrations of impermeable anions with constant average charge do not affect the steady state Cl- gradient, but can shift the cellular volume.** (**A-B**) Different intracellular starting concentrations of impermeable anions do not affect the final steady state concentrations of any ions (top panels: **A** shows [Az]i only; **B** shows ECl in blue, Vm in black and EK in green over varying initial [Az]i), but does cause differences in the steady state volume (bottom panels) linearly proportional to [Az]i (**B**). (**C**) An influx of impermeant anions at a constant rate of the same average charge *z* as the intracellular Az causes transients shifts in ECl (blue, top panel), Vm (black), EK (green) and [Az]i (magenta, bottom panel) for the duration of the influx, and sustained increases in volume (black, middle panel). (**D**) Similarly, the addition of extracellular impermeant anions in an osmo-neutral manner and without affecting [Az]i causes transient shifts in the permeable ion gradients (top panel, colours as in **C**), and sustained changes in cellular volume (black, middle panel) as well as the extracellular Az concentration.

Figure 5

**Changes in the concentrations of impermeable anions that also result in shifts in average charge may affect ionic homeostasis, with different, mechanism-dependent effects on volume.** (**A**) Decreasing the charge of a certain species of impermeant anions from -0.7 to -1, that is decreasing *z* (bottom panel) without changing the total concentration [Az]i, causes persistent depolarsations in ECl (blue, top panel), Vm (black) and EK (green) with moderate increases in volume (middle panel). (**B**) The parametric-analytic solution including KCC2 (solid lines) for varying *z* predicts time series runs based on the mechanism in **A** (dots) in all parameters: ECl, Vm and EK (colours as in **A**, top panel); [Az]i (second-to-top panel); volume (third-to-top panel); and the driving force of chloride DF (bottom panel). The vertical dashes indicate the state at default *z*,-0.85. (**C**) An influx of a species of impermeant anions with charge -1, that is decreasing *z* (bottom panel) and increasing [Az]i, causes persistent depolarsations in ECl, Vm and EK (colours as in **A**, top panel) with large increases in volume (middle panel). (**D**) Repeating the mechanism in **C** for species with different charges *zflux* causes massive changes in volume for small shifts in the average intracellular charge.