Supplemental Data

Supplemental Text S1

Recent structure analysis on the bacterial KtrAB potassium transporter provides direct insights into ion selectivity of this ion channel class (Vieira-Pires et al., 2013). In the channel subunit KtrB, which represents a non-selective member of the Trk/Ktr/HKT family, one potassium ion appears to be coordinated by two “carbonyl oxygen rings” in a single ion binding site in the pore. This potassium coordination structure, which derives from the amino acids preceding the conserved glycine residues, i.e. 111T-115T, 220I-224G, 320T-324A, and 437N-441L, is much shorter as was proposed in earlier models based on structure data of the rather different KcsA (Tholema et al., 2005). In contrast to this previous model, the central glycine residues present in all four pore-lining loops of HKT-type channels seem not directly involved in metal ion coordination, but may rather provide the structural basis for cation selectivity by maintaining the geometry of one of the two carbonyl rings. Together, these two carbonyl rings provide a body-centred cubic-like metal coordination site with a coordination number of 8, considered an ideal coordination geometry for the binding of potassium (see also Fig. 4D). However, due to the inherent asymmetrical pore structure (one chain and not composed of four identical subunits), the carbonyl oxygen-potassium distances are not of identical length. For instance in KtrB, the lower carbonyl ring and the upper carbonyl ring exhibit an average oxygen-metal ion distance of about 2.8 Å and 2.6 Å respectively, but individual carbonyl oxygen-potassium distances vary from 2.2 Å to 3.1 Å. This lack of stringent symmetry/identical metal-carbonyl distances, together with the fact that only one double carbonyl ring exists in HKT-like ion channels (in contrast to strictly K^+-selective KcsA-type ion channels where four such double carbonyl coordination centers exist) may explain the lower K^+/Na^+ selectivity. Concordantly, HKT-type ion channels with four central glycine residues usually transport both alkali ions; they seem incapable of discriminating between Na^+ and K^+. The effect of the ion selectivity upon mutation of one of the four central glycine residues (here in the first pore loop, to a serine) could thus be explained by their impact on the backbone geometry of the preceding residues.

These four glycine residues seem vital for the proper backbone conformation, hence, the geometry of the upper metal-coordinating carbonyl ring (Fig. S3). Consistently, all four central glycine residues adopt backbone torsion angles in KtrB (PDB entry 4J7C), which are not attainable to non-glycine residues because they would cause energetically unfavourable interactions between the main and side chain atoms. If ion selectivity in the HKT family were solely based on the special backbone torsion angle requirement of glycine residues, mutation of glycine at position 84 to any other non-glycine amino acid would yield an ion channel as selective for Na^+ as that of wild-type DmHKT1. However, the mutant S84A still transports K^+, albeit with a K^+ flux only half that observed for S84G (Fig. 4F). Thus, the presence of the four central glycine residues in the pore is important for the formation of an ideal K^+ binding site, which due to its inherent limitations - only one carbonyl double ring and asymmetry of the carbonyl groups forming the metal coordination site - is not fully selective for potassium, but can also bind sodium ions. Exchange of the first pore glycine by small non-glycine amino acid types seems to specifically impair K^+ binding. The mutation-enforced change in backbone conformation of the first pore loop leads to reorientation of the carbonyl group preceding the glycine, thereby resulting in an ion-binding site with a coordination number lower than 8; non-optimal for potassium.

Tholema, N., Vor der Bruggen, M., Maser, P., Nakamura, T., Schroeder, J.I., Kobayashi, H., Uozumi, N., and Bakker, E.P. (2005). All four putative selectivity filter glycine residues in KtrB are essential for high affinity and selective K(+) uptake by the KtrAB system from *Vibrio alginolyticus*. J Biol Chem 280:41146-41154.

Vieira-Pires, R.S., Szollosi, A., and Morais-Cabral, J.H. (2013). The structure of the KtrAB potassium transporter. Nature 496:323-328.

Supplemental Figs

S1

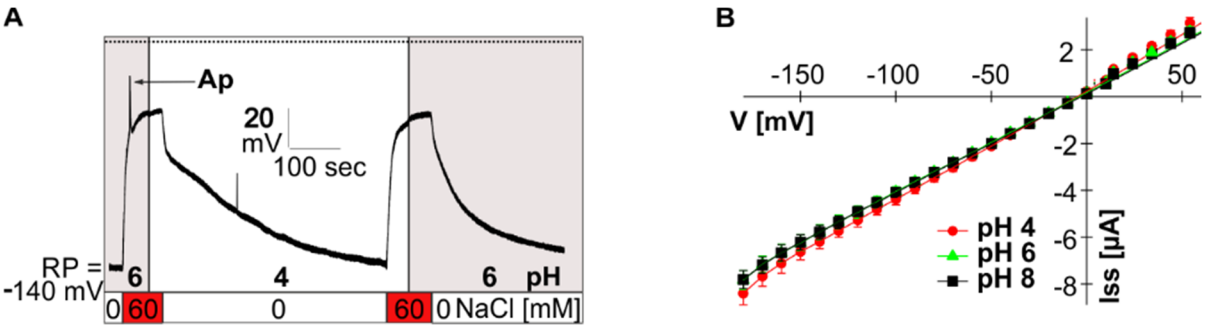

Fig. S1 related to Fig. 2

(A): Sodium-dependent depolarization of digesting *Dionaea* glands is H^+ independent. Changing the pH from 6 (grey background) to 4 (white background) and *vice versa* did not influence the depolarization caused by the addition of 60 mM NaCl (red bar). Na^+-dependent depolarization often resulted in the spontaneous firing of APs, as indicated. (B): Recorded steady-state currents (I_{SS}) and reversal potentials of DmHKT1-expressing oocytes in 100 mM NaCl containing bath solution were not influenced by an external pH change from 8 to 6 and 4 (n = 4, mean ± SD).

S2

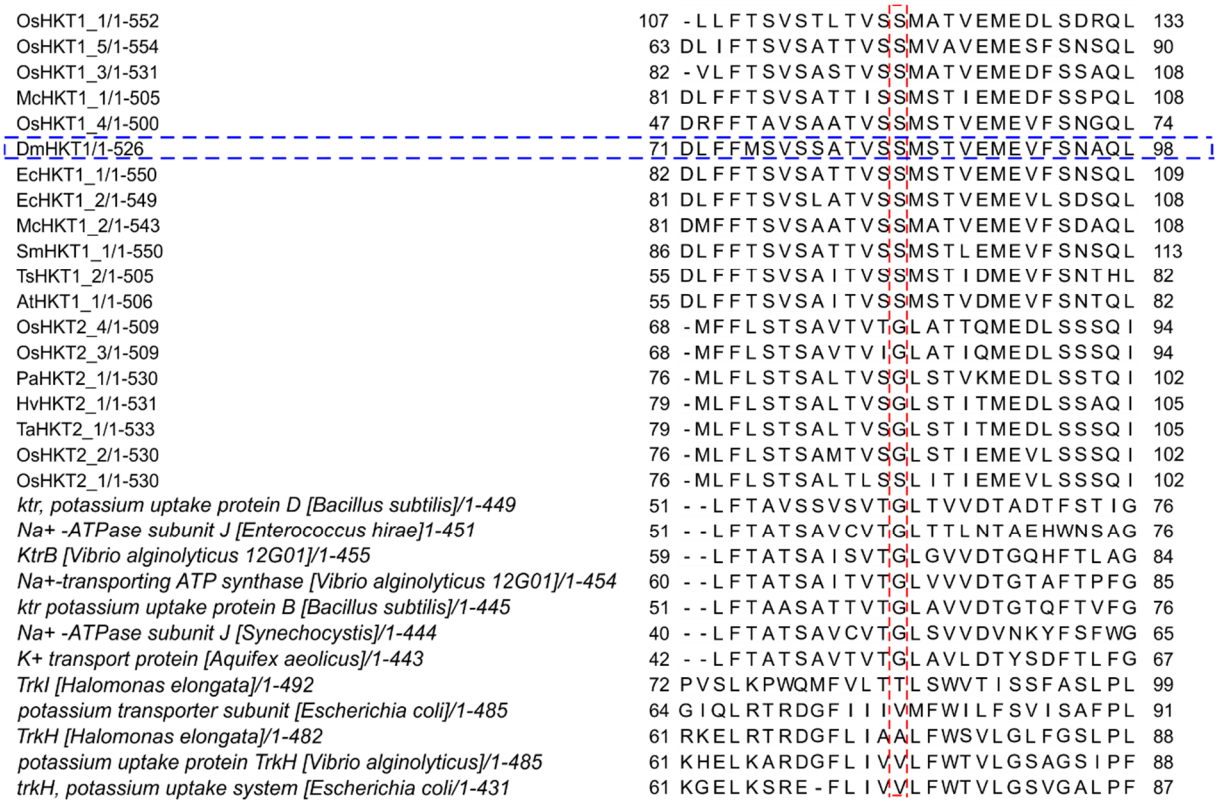

Fig. S2 related to Fig. 3

Sequences of Trk/Ktr/HKT orthologs from various plant, bacterial and fungal species were aligned by ClustralW2. HKT1 from *Dionaea muscipula* is boxed in blue. The selectivity filter in the 1st P-loop is marked in red (jalview 2.7).

S3

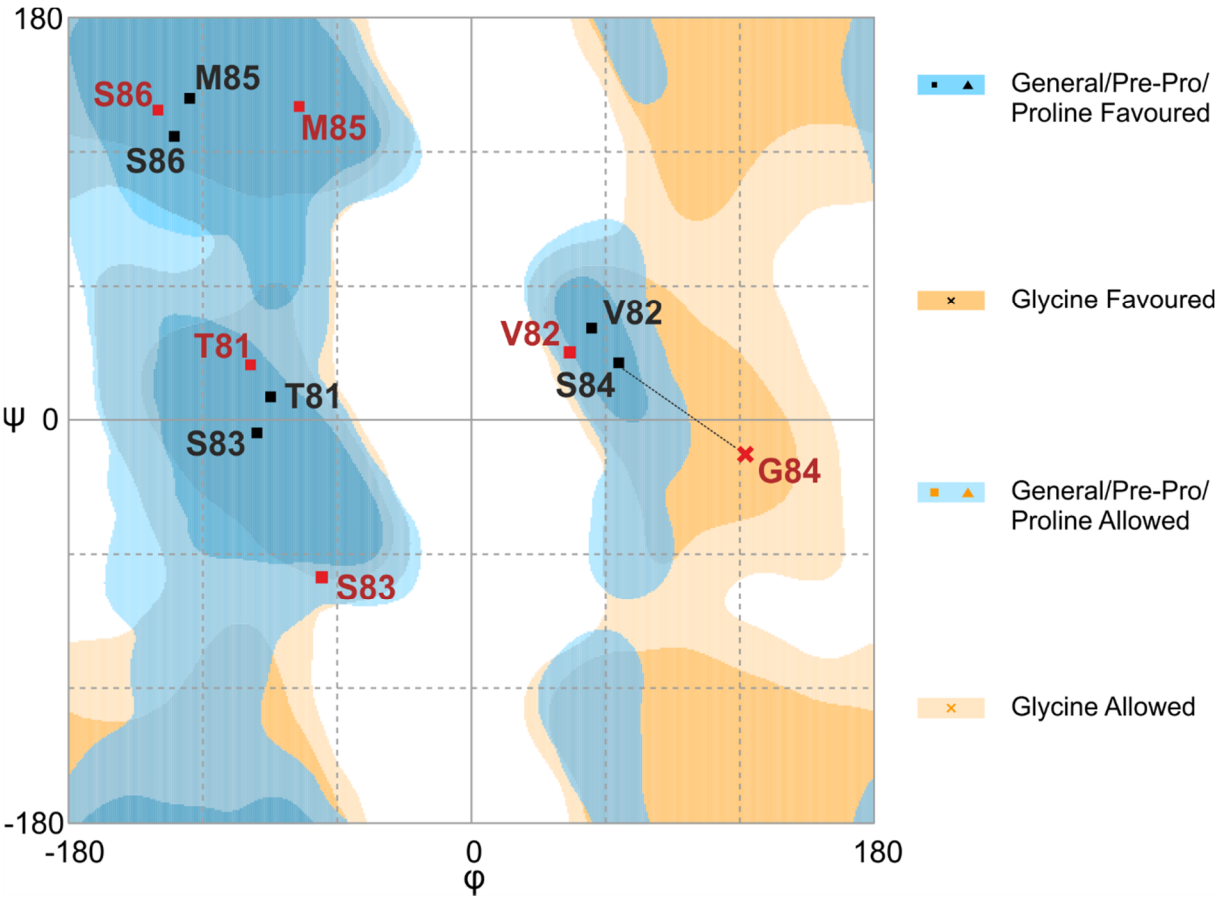

Fig. S3 related to Fig. 4

Backbone torsion angle (Ramachandran) analysis for the residues 81 to 86 of the pore-lining loop 1 of DmHKT1. Shown is the Ramachandran plot for the pore loop of wild-type DmHKT1 (black squares and crosses) and the mutant S84G (red squares and crosses). In the mutant, the glycine residue (G84) at the central pore position adopts backbone torsion angles, which are also found for the equivalent glycine residue in the pore loops of the bacterial homologs KtrB and TrkH. However, this phi/psi-torsion angle combination (KtrB G62 φ = 122°, ψ = -15°; TrkH G113 φ = 109°, ψ = 26°; DmHKT1 G84 φ = 123°, ψ = -15° is only accessible to glycine residues (orange coloured areas) and cannot be achieved by any other amino acid type due to steric (and energetic) restraints. Thus, upon replacement of this glycine residue by a serine, as found in DmHKT1, the backbone torsion angles of Ser84 requires a change in the phi as well as the psi-angle to adopt an energetically favourable conformation (here Ser84 is located in the area characteristic for left-handed helices (blue area)). However, a manual remodeling of the backbone geometry to accommodate favourable main chain

torsion angles for Ser84 (φ = 66°, ψ = 26°) not only dislocates the carbonyl group of the preceding Ser83 by 2.9Å (backbone torsion angles change from φ = -95°, ψ = -6° in wild-type DmHKT1 to φ = 67°, ψ = -70° in S84G), but also leads to further changes in the pore loop backbone such that the carbonyl group of Val82 is also moved away (by 1.3Å) from the ion coordination centre. This means that its capacity to coordinate the metal ion might be limited.