Extended experimental procedures

Generation of the model of a hydrated voltage-sensor

The hydrated model of the voltage-sensor was generated using molecular dynamics simulations, performed using NAMD (Phillips et al., 2005) with CHARMM 36 force-field parameters. The VSD of the K_V 1.2/2.1 paddle-chimera structure (PDB-ID: 2R9R, residues 159-310, along with crystallographic waters within 5 Å of the aforesaid region of the protein) was embedded in a POPC membrane, with additional layers of water molecules bathing the bilayer using VMD. After energy minimization (1ns), a 2ns equilibration run was performed during which a harmonic constraint (of spring constant, 1 kcal/mol/$Å^2$) was applied to the protein atoms and the crystallographic water molecules, while the remaining water molecules were kept outside of the membranous space using user defined forces. This allows melting of lipid tails. Subsequently, in a second equilibration run of 2ns, all constraints were eliminated. Next, a 5 ns production run was performed in an NPT ensemble, with the area in the x-y plane maintained constant over the duration of the simulation. In all steps of the simulation, the temperature was maintained at 25°C, periodic boundary conditions were imposed, the time-step of simulation was 1fs and no external electric field was imposed. The model of the VSD shown in **Fig. 1d**, is a snapshot from the last 1 ns of the production run.

Supplementary Analysis

The contributions of different residues to the net ΔCp associated with channel gating can be written as:

$$\text{Net } \Delta C_P = \Delta C_{P(+)} + \Delta C_{P(-)} + \Delta C_{P(0)}$$

where $\Delta C_{P(+)}$ and $\Delta C_{P(-)}$ denotes the contribution of amino acids which respectively increase or decrease the ΔCp during channel opening. $\Delta C_{P(0)}$ represents all the other amino acids which do not contribute to the ΔC_P. Since the wild type Shaker K+ channel does not exhibit any temperature-dependent shift in its relative open probability, the net ΔC_P associated with its gating is likely to be zero. Coupled with our current understanding of structural dynamics of the protein (Jensen et al., 2010; Jensen et al., 2012), studies measuring osmotic and pressure effects on channel gating (Schmidt et al., 2012; Zimmerberg et al., 1990) indicate that channel opening is associated with increase in water-accessible surface area of the protein. Since the change in solvent accessible surface area of the protein is associated with a change in ΔC_P, a zero net ΔC_P of Shaker channel gating can arise if $\Delta C_{P(+)}$ and $\Delta C_{P(-)}$ components are balanced.

We consider a site which undergoes solvation during channel activation which natively was responsible for $\Delta C_{P(0)}$. When such a site is occupied by a polar residue, it solvation in the activated state will lead to a negative ΔC_P and lead to heat sensitivity (**Fig. S1a**). Conversely when the site harbors a non-polar residue, its solvation will lead to a positive ΔC_P and thus cold sensitivity (**Fig. S1a**). This would be the case for our 293 site.

A different scenario arises when we consider a site, which undergoes solvation when the channel activates and contributes to $\Delta C_{P(+)}$ component. At such a site, a non-polar residue (whose solvation is associated with a positive ΔC_P) will not alter the net ΔC_P of channel gating and the channel will remain temperature insensitive. However, replacing a polar residue at such a side will reduce the $\Delta C_{P(+)}$ component which means that the net ΔC_P is dominated by $\Delta C_{P(-)}$ term, effectively making the process in heat sensitive (**Fig. S1b**). In both the scenarios, polar residues at the two sites lead to heat sensitivity, however increasing the hydrophobicity in the first case causes a switch in the temperature sensing phenotype of the channel while in the second case, and it simply compromises the heat sensing phenotype of the channel.

An additional point that is noteworthy is that polar residues in general exhibit smaller magnitudes of ΔC_P that their hydrophobic counterparts (Makhatadze and Privalov, 1990). Some simulation studies (using specific force fields/water models) (Sedlmeier and Netz, 2013) have suggested that polar residue solvation, depending on the local environment, may exhibit even slightly positive ΔC_P. Despite such a caveat, a polar substitution in place of a hydrophobic site in the wild type Shaker channel undergoing solvation will result in a net negative ΔC_P, as it removes the positive contributions of the hydrophobic moiety. Conversely, a polar substitution at a hydrophobic site undergoing desolvation will confer a net positive ΔC_P by attenuating the negative ΔC_P conferred by hydrophobic site desolvation.

Table S1. Effect of mutations on the relative P_OV curves at two temperatures in Shaker potassium channels.

Mutant	V_M (s.e.) 28°C (in mV)	n	V_M (s.e.) 8°C (in mV)	n	ΔV_M (s.e.) (in mV)	z_{HT} (s.e.) / z_{LT}(s.e.)
WT	-20.6 (1.7)	4	-15.9 (2.1)	5	-4.7 (2.7)	2.83 (0.14) / 2.08 (0.15)
S1-S3 crevice facing mutants						
S233I	×		×		×	×
S233V	21.8 (2.9)	6	21.1 (3.9)	7	0.7 (4.9)	1.17 (0.05) / 1.04 (0.04)
S233D	16.6 (1.5)	4	9.1 (4.6)	4	7.5 (4.8)	1.29 (0.09) / 1.16 (0.01)
S240I	×		×		×	×
S240V	-8.2 (1.2)	5	-13.3 (2.7)	4	5.1 (2.9)	3.44 (0.3) / 2.06 (0.20)
S240A	-6.9 (1.4)	5	5.2 (2.0)	4	-12.1 (2.4)	1.36 (0.09) / 1.35 (0.07)
S240H	118.7 (1.4)	4	105.1 (1.2)	4	13.6 (1.8)	1.35 (0.04) / 1.41 (0.06)
S240D	131.0 (5.0)	4	130.0 (1.4)	3	1.0 (5.2)	1.48 (0.12) / 1.07 (0.05)
E283I	×		×		×	×
E283V	×		×		×	×
E293I	-3.6 (2.7)	4	-21.5 (1.7)	8	17.9 (3.2)	1.42 (0.08) / 1.57 (0.03)
E293A	-13.6 (0.7)	4	-4.0 (1.9)	4	-9.6 (2.0)	1.70 (0.16) / 1.25 (0.05)
E293Q	0.2 (2.6)	6	13.5 (2.0)	5	-13.3 (3.3)	1.81 (0.19) / 1.66 (0.15)
E293H	-27.6 (0.6)	4	-3.4 (1.3)	4	-24.2 (1.4)	1.87 (0.02) / 1.33 (0.11)
N313V	3.3 (4.0)	6	0.8 (4.2)	6	2.5 (5.8)	1.28 (0.08) / 1.18 (0.08)
N313D	-15.7 (1.9)	4	-16.3 (3.3)	5	0.6 (3.8)	2.32 (0.22) / 1.44 (0.04)
D316I	×		×		×	×
D316V	×		×		×	×
Y323I	-9.9 (1.3)	4	21.6 (2.4)	4	-31.5 (2.7)	2.49 (0.06) / 1.02 (0.03)
Y323A	-2.4 (0.9)	4	11.2 (3.3)	4	-13.6 (3.4)	2.04 (0.15) / 1.69 (0.25)
Y323T	25.8 (1.7)	6	15.1 (1.2)	5	10.7 (2.1)	1.20 (0.09) / 1.30 (0.04)
Y323Q	40.8 (1.6)	6	29.3 (0.9)	5	11.5 (1.8)	1.23 (0.06) / 1.17 (0.04)
T326I	-30.7 (1.7)	5	-32.0 (1.6)	5	1.3 (2.3)	1.79 (0.19) / 1.93 (0.25)
T326N	-18.4 (2.0)	5	-18.1 (2.0)	5	-0.3 (2.8)	1.85 (0.27) / 1.36 (0.06)
Hydrophobic residues in S4						
I6	0.1 (1.4)	9	0.0 (2.7)	10	0.1 (3.0)	1.68 (0.05) / 1.33 (0.08)
M6	-27.2 (1.5)	4	5.6 (2.8)	4	-32.8 (3.2)	2.17 (0.29) / 0.91 (0.05)
A6	-69.8 (1.7)	4	-33.6 (2.0)	4	-36.2 (2.6)	3.74 (0.11) / 1.11 (0.02)
I4	11.2 (2.4)	5	10.2 (3.0)	4	1.2 (3.8)	1.23 (0.05) / 1.05 (0.09)
M4	38.7 (1.5)	6	52.9 (1.9)	4	-14.2 (2.4)	1.21 (0.03) / 0.95 (0.01)
A4	-84.9 (1.7)	3	-55.8 (4.7)	4	-29.1 (5.0)	2.98 (0.08) / 1.37 (0.12)
I2Top	-4.4 (1.7)	7	-18.9 (1.9)	12	14.5 (2.5)	1.45 (0.15) / 1.53 (0.09)
M2Top	50.8 (1.4)	5	31.9 (1.0)	5	18.9 (1.7)	1.33 (0.04) / 1.3 (0.07)
A2Top	-5.4 (3.0)	7	-7.0 (3.7)	4	1.6 (4.8)	1.23 (0.08) / 1.25 (0.07)
S2Top	-10.2 (0.8)	3	10.6 (0.7)	3	-20.8 (1.1)	1.91 (0.09) / 1.19 (0.03)
I2Mid	23.2 (2.8)	4	26.5 (2.6)	5	-3.3 (3.8)	1.16 (0.04) / 1.16 (0.02)
M2Mid	-4.5 (2.8)	5	8.9 (1.9)	5	-13.4 (3.4)	1.52 (0.08) / 1.46 (0.07)
A2Mid	-26.2 (2.2)	5	-36.2 (0.6)	4	10.0 (1.9)	1.79 (0.18) / 1.45 (0.55)
S2Mid	-40.1 (1.4)	7	-29.5 (2.6)	4	-10.6 (3.0)	1.75 (0.14) / 1.28 (0.01)
I2Bot	-16.7 (2.2)	5	0.6 (1.8)	4	-17.3 (2.8)	2.18 (0.39) / 1.09 (0.08)
M2Bot	-12.1 (2.8)	4	15.0 (3.5)	7	-27.1 (4.5)	1.65 (0.18) / 0.98 (0.04)
A2Bot	31.3 (0.9)	4	17.3 (1.6)	4	14.0 (1.8)	1.06 (0.09) / 1.32 (0.07)
S2Bot	-17.4 (1.7)	5	51.1 (2.4)	4	-68.5 (2.9)	4.49 (0.39) / 1.21 (0.04)
Combination mutants						
Y323I/S2Mid	-46.3 (1.9)	3	8.1 (2.5)	5	-54.4 (3.1)	3.97 (0.72) / 1.08 (0.06)
Y323I/M6	32.4 (2.4)	8	70.5 (2.7)	7	-38.1 (3.6)	1.04 (0.04) / 0.78 (0.02)
S4 charge mutants						
R362A	24.9 (1.5)	6	23.5 (2.1)	7	1.4 (2.6)	1.23 (0.02) / 1.18 (0.02)
R362Q	19.8 (1.9)	5	17.1 (4.6)	5	2.7 (5.0)	1.33 (0.10) / 1.16 (0.17)
R365A	19.9 (1.9)	7	9.3 (3.2)	6	10.6 (3.7)	1.58 (0.06) / 1.38 (0.17)
R365Q	-19.0 (0.6)	4	-21.3 (3.6)	3	2.3 (3.6)	1.52 (0.09) / 1.27 (0.02)
R368A	50.7 (3.2)	7	47.9 (2.8)	5	2.8 (4.3)	0.8 (0.04) / 0.84 (0.05)
R368N	-24.1 (2.6)	4	-31.6 (3.5)	3	7.5 (4.4)	1.63 (0.12) / 1.84 (0.20)
R371A	-50.3 (1.8)	11	-45.1 (1.7)	4	-5.2 (2.5)	2.69 (0.24) / 1.52 (0.3)
R371Q	-12.0 (1.8)	8	-13.5 (5.2)	3	1.5 (5.5)	1.38 (0.07) / 1.26 (0.14)
Y323I/R368N	-14.3 (2.3)	5	40.3 (1.1)	4	-54.6 (2.5)	1.94 (0.12) / 0.91 (0.06)
Y323I/R371Q	-15.1 (2.1)	6	60.3 (6.9)	4	-75.4 (7.2)	2.78 (0.24) / 0.81 (0.03)
S2Mid/R371Q	-80.2 (1.1)	3	-57.0 (0.8)	4	-23.2 (1.3)	5.28 (0.71) / 3.29 (0.27)
Y323Q/R371Q	-3.0 (1.6)	6	-27.4 (2.9)	3	24.4 (3.3)	1.58 (0.06) / 1.56 (0.11)

The table lists the median voltage of channel opening (V_M) of each of the mutants of the Shaker K_V channel at 28°C and 8°C (except for Y323I/M6, where the V_M values are those at 28°C and 15°C). The standard error associated with each V_M is reported in parenthesis. 'n' indicates the number of replicates (independent measurements in oocytes) used to obtain the V_M at each temperature. ΔV_M is the change in V_M between the high and the low temperature (ΔV_M = $V_M(28°C) - V_M(8°C)$, or for the Y323I/M6 mutant: $\Delta V_M = V_M(28°C) - V_M(15°C)$). The standard error associated with ΔV_M, denoted as $\delta\Delta V_M$, was calculated as: $\sqrt{\delta V_M^2(28°C) + \delta V_M^2(8°C)}$, where δV_M is the standard error associated with each V_M measurement. The last column reports the steepness of the relative $P_O V$ curves at the high and low temperatures (z_{HT} and z_{LT} respectively), with their respective standard errors in parenthesis. The steepness of each curve is its 'Boltzmann slope' obtained by fitting a Boltzmann equation to the curve. Mutants were classified as temperature sensitive if $|\Delta V_M| > 3\,\delta\Delta V_M$ and are colored red or blue depending on whether they are heat or cold sensitive respectively. In many instances the activation curves of the mutants were not adequately fitted by a Boltzmann equation and required superposition of multiple Boltzmann components. To enable a straightforward comparison of the temperature dependent shifts occurring with different mutants, the V_M was used as it is obtained by a direct integral transformation of the activation curve and not by fitting the activation curves to pre-constrained multi-parametric equations (Chowdhury and Chanda, 2012). Additionally, it is important to remember that the change in the 'Boltzmann slope' values with temperature reflect the change of 'macroscopic voltage-dependence', which arises chiefly due to the stability of the intermediate states of activation of the channel (Schoppa et al., 1992).

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Supplementary Figure Legends

Supplementary Figure S1. Polarity correlated ΔG *vs* T profiles for different perturbations at a site undergoing solvation during channel activation and temperature sensitivity of the wild-type Shaker K_V channel (Related to Figure 1). (A) At a site which in the native channel contributes to $\Delta Cp_{(0)}$, polar perturbations lead to heat sensitivity (gray region) and increasing the hydrophobicity progressively renders the channel cold sensitive **(B)** At a site, which in the native channel contributes to $\Delta Cp_{(+)}$, residues of relatively high polarity causes heat sensitivity while those of relatively high hydrophobicity attenuate the heat sensitivity but do not necessarily switch the temperature sensing phenotype of the channel. **(C, D)** Representative ionic currents at 28°C **(C)** and 8°C **(D)** from an oocyte expressing wild-type Shaker K_V channel, elicited by depolarizing voltage pulses from -60 to +60mV, in 10mV increments, from a holding voltage of -120mV.

Supplementary Figure S2. Temperature-sensitivity of crevice facing sites in S1-S3 (Related to Figure 2). Representative ionic currents at 28°C **(red)** and 8°C **(blue)** from single oocytes expressing mutant channels, elicited by depolarizing voltage pulses in 10mV increments ($V_{Holding}$ = -120mV). Scale bars represent 5 μA (except **(F)** for which it is 10 μA) and 50 ms. In each set of traces, the arrow points to the current response at the indicated voltage. The mutants corresponding to these raw current traces are: S240V **(A)**, S240H **(B)**, E293I **(C)**, E293H **(D)**, Y323I **(E)** and Y323Q **(F)**. **(G)** Normalized conductance *vs* voltage curve at 28°C (red) and 8°C (blue) for hydrophobic perturbations (top row) and polar perturbations (bottom row) at sites S233 (left), N313 (center) and T326 (right). **(H)** For three sites in the external crevice, T326, Y323 and S240, the polarity of the perturbation is plotted against the V_M, at 28°C, of each mutant. Arrows connect the two perturbations at the same site (written alongside the arrow) and indicate the change in V_M in response to a change in polarity. As indicated, increase in polarity shifts the V_M rightward in all three cases. Polarity scale is the same as described in Figure 2. **(I)** Same as **(B)**, except that three sites, S233, E293, N313, reside in the internal crevice – for each of these sites increase in polarity shifts the V_M leftward.

Supplementary Figure S3 (Related to Figure 3). Perturbations of the hydrophobic residues in S4 alter temperature sensing phenotype. (A), (B) The V_M (at 28°C) and ΔV_M due to a 20° change in temperature for the hextuplet **(A)** and quadruplet **(B)** mutations to Ile, Met and Ala are represented as bar graphs. The hydrophobicity order of the perturbations is Ile>Met>Ala. **(C)-(K)** Normalized conductance *vs* voltage curve at 28°C (red) and 8°C (blue) for doublet perturbations – top row **(C)-(E)** Ile perturbations, middle row **(F)-(H)** Met perturbations and bottom row **(I)-(K)** Ala perturbations. The first column (**(C), (F) and (I)**) represent the top doublets, the second (**(D), (G) and (J)**) and the third (**(E), (H) and (K)**) columns represent the middle and bottom doublets respectively. **(L)-(N)** Representative ionic currents at 28°C **(red)** and 8°C **(blue)** from single oocytes expressing mutant channels, elicited by depolarizing voltage pulses in 10mV increments ($V_{Holding}$ = -120mV). In each set of traces, the arrow points to the

current response at the indicated voltage. The mutants corresponding to these raw current traces are: the hextuplet Ala mutant, A6 **(L)**, the quadruplet Ala mutant, A4 **(M)** and the bottom doublet Ser mutant, S2Bot **(N)**. Scale bars represent 2 μA and 50 ms.

Supplementary Figure S4 (Related to Figure 5). Temperature-sensitivity due to gating charge neutralizations in the wild type background. Normalized conductance *vs* voltage plots at 28°C (red) and 8°C (blue) for R362A **(A)**, R362Q **(B)**, R365A **(C)** and R365Q **(D)**.