Supplementary Information for

# A Folding Reaction at the C-Terminal Domain Drives Temperature Sensing in TRPM8 Channels

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## Supplementary Methods

*Electrode preparation.* Pipettes of borosilicate capillary glass (World Precision Instruments) were pulled on a horizontal pipette puller (Sutter Instruments) and fire polished using a Narishige microforge MF-830.

*Macroscopic currents recording and analysis*. Voltage protocols for experiments in Fig. 1 were as in (1). For experiments in Figs. 2 and 3 the membrane was held at -120 or -90 mV for 2-5 ms followed by a depolarization to different test voltages starting from the holding potential to 420 mV in 10- or 20-mV increments (Fig. 1) and 5-mV (Fig. 2 and 3). To compute Conductance vs Voltage (G-V) relations the tail currents were measured at the end of the test pulse applying a pulse of 180 mV or 250 mV as indicated in the Figure legends. The pulse protocol ends with a final closing epoch to the holding potential during 3-5 ms. Capacitive currents were compensated using a P/−4 subtracting protocol from the holding potential (2).

The G/ $G_{max}$ data were obtained from the normalized tail currents amplitude acquired at the "tail epoch" that follow the test pulse. The G/ $G_{max}$ vs. Voltage curves were fitted using a Boltzmann function:

$$I_{tail}(V) = \frac{I_{max}}{1+e^{\frac{zF}{RT}*(V_o-V))}} \quad (1)$$

where $I_{max}$ is the maximal current predicted by the fit, *Vo* is the half-activation voltage, z is the slope of the curve and F,R and T have their usual meanings.

In Figures 2-3 some G/Gmax vs. Voltage curves do not saturate. To determine the error in the Boltzmann fit parameters for a non-saturating G-V curve, we truncated a saturating G-V curve, generating several truncated variants and we fit these truncated datasets to the Boltzmann equation. We estimated that performing a Boltzmann function fit with data sets whose Gmax ≥0.70 leads to an 11.9% error in the fit parameters as compared with those obtained with a Gmax= 1, an error we considered acceptable.

*Voltage Protocol.* Test pulse durations were different in each experimental series ranging from 2 to 50 ms. This was due to that in some conditions the patch becomes very unstable, which increases with voltage and temperature requiring pulses of short duration in particular in the presence of urea and at high applied voltages. All voltage steps and their respective durations are detailed in Supplementary Table III. Comparison between experiments obtained using the different voltage protocols are valid given that: i) membrane currents develop above 80% of its steady-state value determined after fitting the current time course to an exponential function. ii)

G/Gmax vs V data saturate or is close to saturation and are reasonably well fitted to a Boltzmann function.

Table S1. **Van´t Hoff Equation fit parameters of Figure 1E**

| channel | $\Delta H_1$ (kcal/mol) | $\Delta S_1$ (cal/molK) | $\Delta H_2$ (kcal/mol) | $\Delta S_2$ (cal/molK) |
|---|---|---|---|---|
| TRPM8 wt | -61±4 | 0.21±0.01 | -18±2 | 0.07±0.01 |
| TRPM8ΔCT8 | -43.5±3 | 0.15±0.01 | -21.7±10 | 0.08±0.03 |
| TRPM8ΔCT15 | -33.6±2 | 0.12±0.06 | -26.3±6 | 0.100±0.023 |
| TRPM8ΔCT36 | -21.4±2 | 0.08±0.03 | -4±5 | 0.020±0.008 |

Table S1. Enthalpy and Entropy changes obtained after fitting the lnK obtained from experiments in Figure 1C vs temperature plot to the Van´t Hoff equation. Columns shows (from left to right) TRPM8 channel variant, enthalpy and entropy changes during channel gating at the high ($\Delta H_1$, $\Delta S_1$ ) and low ($\Delta H_2$ , $\Delta S_2$ ) temperature-dependent regime.

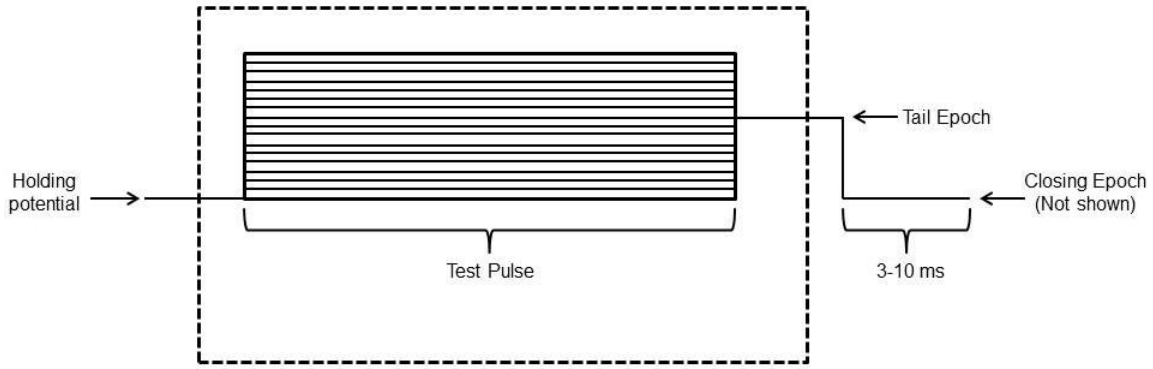
**Table S2.** Boltzmann fit parameters of Experiments in Figure 2&3

| channel | condition | temperature | Vo | z | N |
|---|---|---|---|---|---|
| TRPM8 | control | 10°C | 103.7±7 | 0.50±0.02 | 5 |
| TRPM8 | control | 25°C | 234±7 | 0.50±0.04 | 5 |
| TRPM8 | Urea 3M | 10°C | 290.7±7.5 | 0.40±0.01 | 4 |
| TRPM8 | Urea 3M | 25°C | 255.7±9 | 0.80±0.10 | 4 |
| TRPM8 | Sucrose 2M | 10°C | 309±10 | 0.45±0.03 | 5 |
| TRPM8 | Sucrose 2M | 25°C | 319±10 | 0.60±0.05 | 5 |
| | | | | | |
| TRPM8ΔCT36 | control | 10°C | 319±8 | 0.76±0.03 | 4 |
| TRPM8ΔCT36 | control | 25°C | 334±4 | 0.66±0.05 | 6 |
| TRPM8ΔCT36 | Urea 3M | 10°C | 384.5±7 | 0.50±0.01 | 4 |
| TRPM8ΔCT36 | Urea 3M | 25°C | 328±7 | 0.70±0.05 | 5 |
| TRPM8ΔCT36 | Sucrose 2M | 10°C | 344±5 | 0.70±0.05 | 4 |
| TRPM8ΔCT36 | Sucrose 2M | 25°C | 321±4 | 0.60±0.04 | 7 |

Table S2. Boltzmann fit parameters for experimental series of Figure 2 and 3. Columns shows (from left to right) TRPM8 channel variant, internal recording solution, temperature, half-voltage of maximal activation Vo, z the apparent effective valence and number of experiments per condition.

**Table S3.** Detailed Voltage protocols of Experiments in Figure 2&3

A




B

| TRPM8 wt | | | | | TRPM8ΔCT36 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Epoch** | **HP** | **Test pulse (max)** | **Tail Epoch** | **Closing Epoch** | **Epoch** | **HP** | **Test pulse (max)** | **Tail Epoch** | **Closing Epoch** |
| **10 ºC control** | | | | | **10 ºC control** | | | | |
| **Time (ms)** | 5 | 20 | 5 | 10 | **Time (ms)** | 2 | 20 | 10 | 5 |
| **V (mV)** | -140 | 235 | 190 | -140 | **V (mV)** | -90 | 420 | 200 | -90 |
| | | | | | | | | | |
| **25 ºC control** | | | | | **25 ºC control** | 5 | 15 | 5 | 5 |
| **time (ms)** | 2 | 5 | 2 | 5 | **time (ms)** | -90 | 390 | 220 | -90 |
| **V (mV)** | -90 | 345 | 220 | -90 | **V (mV)** | | | | |
| | | | | | | | | | |
| **10 ºC urea** | | | | | **10 ºC urea** | | | | |
| **time (ms)** | 2 | 25 | 5 | 10 | **time (ms)** | 2 | 10 | 2 | 5 |
| **V (mV)** | -100 | 340 | 190 | -100 | **V (mV)** | -90 | 420 | 220 | -90 |
| | | | | | | | | | |
| **25 ºC urea** | | | | | **25 ºC sucrose** | | | | |
| **time (ms)** | 2 | 20 | 5 | 5 | **time (ms)** | 2 | 10 | 2 | 3 |
| **V (mV)** | -100 | 340 | 190 | -100 | **V (mV)** | -90 | 420 | 220 | -90 |
| | | | | | | | | | |
| **10 ºC sucrose** | | | | | **10 ºC urea** | | | | |
| **time (ms)** | 5 | 15 | 5 | 5 | **time (ms)** | 2 | 20 | 10 | 3 |
| **V (mV)** | -90 | 390 | 195 | -90 | **V (mV)** | -90 | 420 | 195 | -90 |
| | | | | | | | | | |
| **25 ºC sucrose** | | | | | **25 ºC sucrose** | | | | |
| **time (ms)** | 5 | 15 | 5 | 5 | **time (ms)** | 2 | 10 | 2 | 5 |
| **V (mV)** | -90 | 370 | 220 | -90 | **V (mV)** | -90 | 380 | 220 | -90 |

Table S3. A) Voltage protocol scheme. It starts with a step at the holding potential (HP) to then run the test pulses to the maximal indicated value (Test pulse max) in 5mV steps. with the test pulse is followed by a “tail Epoch” where the tail current develops and ends when the voltage is returned to the holding potential. Part of the tail Epoch and the closing Epoch were omitted from figures to improve tail currents visualization. B) Detailed protocols for experimental series of Figures 2 and 3. First row indicates the TRPM8 variant, second row shows the different sections of the voltage protocol (HP, Test pulse (max), Tail Epoch and Closing Epoch). Columns 1 and 6 indicate the temperature, duration, and experimental condition (Control, Urea and Sucrose).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2. C. M. Armstrong, F. Bezanilla, Currents related to movement of the gating particles of the sodium channels. *Nature* **242**, 459-461 (1973).