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Supplemental Information

The Contribution of the Ankyrin Repeat Domain of TRPV1 as a Thermal Module

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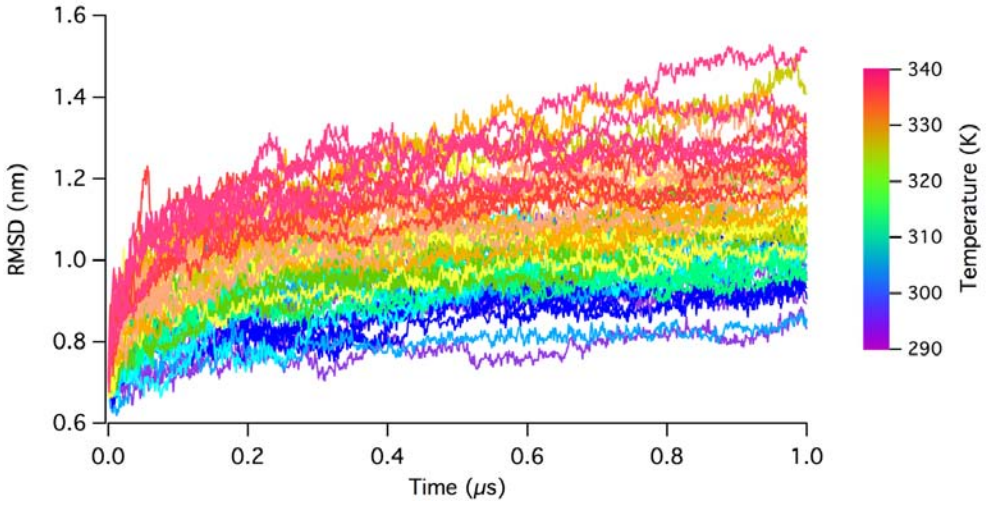
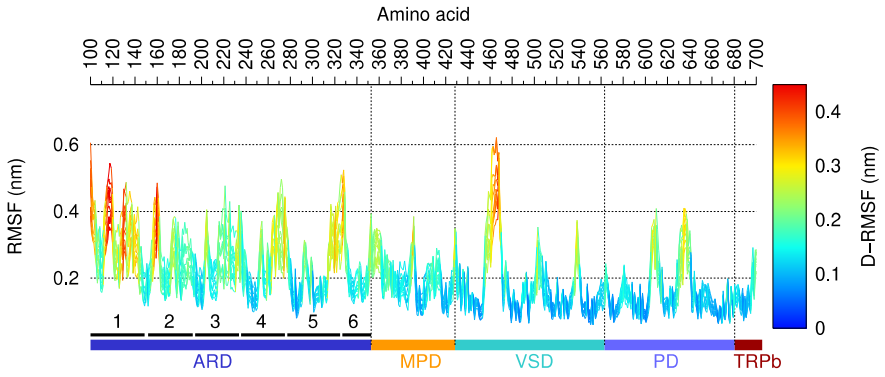
Supplementary figures



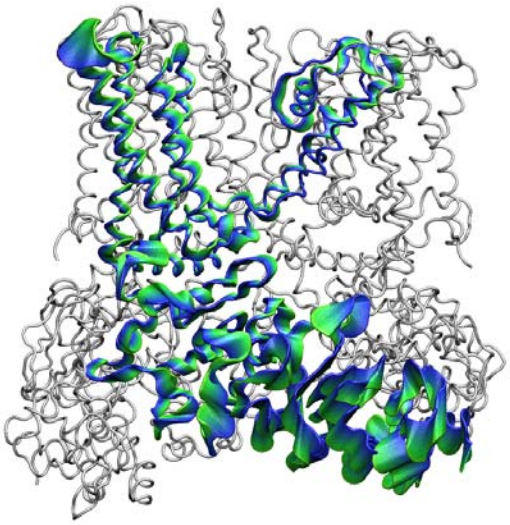
Supplementary figure 1

Supplemental Figure 1. Stability of the TRPV1 structure during simulation. RMSD value of all replicas at all simulated temperatures calculated over the 1 microsecond simulation. The color scale bar indicates the color code representing the simulation temperature. No large structural drifts were observed over the simulation time.



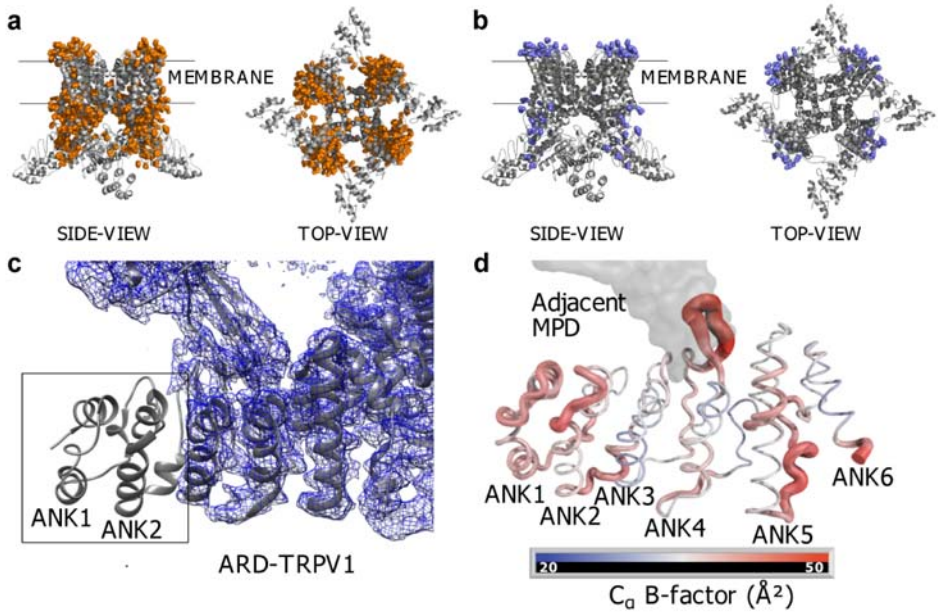
Supplementary figure 2

Supplemental Figure 2. The RMSF values for each simulated temperature, color-coded by the difference between the RMSF values from our highest temperature simulations (340 K) and our coldest simulated TRPV1 model at 290 K. The largest differenced in RMSF are seen in the ARD, especially domains 1 and 2.



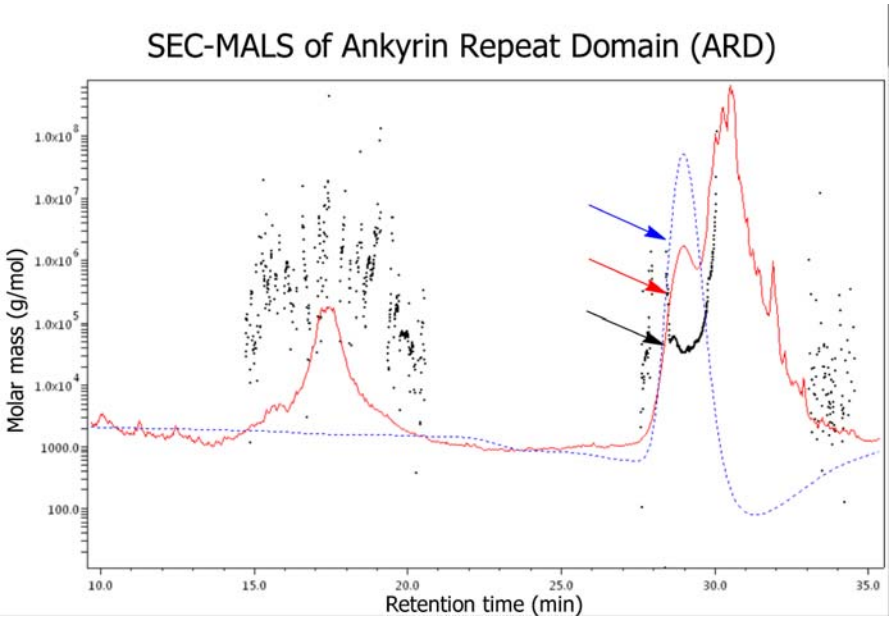
Supplementary figure 3

Supplemental Figure 3. Fluctuations calculated by RMSF per bead relative to the main chain. This figure shows fluctuations of an entire subunit, not only the ARD as in figure 4c. Regions with larger values of fluctuations are represented by the thicker ribbon.



Supplementary figure 4

Supplemental Figure 4. Quality of TRPV1 cryo-EM structures and crystallographic structure of ARD. (a) Side view of the missing occupancies of side chain atoms in the capsaicin-bound (CB) structure, PDBID: 2J5R. Orange spheres indicate the aminoacids where the electronic density is not seen and the heavy atoms are not described; a representation by spheres shows the aminoacids missing in the structure. Bottom view of the CB structure showing missing atoms in the cryo-EM structure. (b) Missing occupancies in the APO structure (3J5P), the missing occupancies vs. the CB structure are represented; the APO structure is a reliable model for coarse-grained MD. (c) Ankyrin motifs 1 (ANK1) and 2 (ANK2) do not show an electronic density in the 2J5P structure, this implies a low stability or high movement even at cryogenic temperatures. The electron density is shown in blue, while the structure of the ARD determined by X-ray diffraction is fitted to the density and show in ribbon representation. (d) Beta-factor representation in putty cartoon of the crystallographic structure of the ARD in solution (PDBID: 2PNN), code color shows red regions have poor density (assumed higher movement) and blue regions have a good density (lower movement).



Supplementary figure 5

Supplemental Figure 5. SEC-MALS of isolated ankyrin repeat domain. Chromatogram obtained in 20 mM glycine, pH 9.5, 200 mM NaCl, 25 °C. The peak (arrows) corresponds to a population with MW = 32.4 kDa and R_g = 3.1 nm. Colors correspond to light scattering (red), refractive index (blue) and molar mass (black).