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Supplemental Information

Decrypting the Heat Activation Mechanism of TRPV1 Channel by Molecular Dynamics Simulation

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Supporting Information

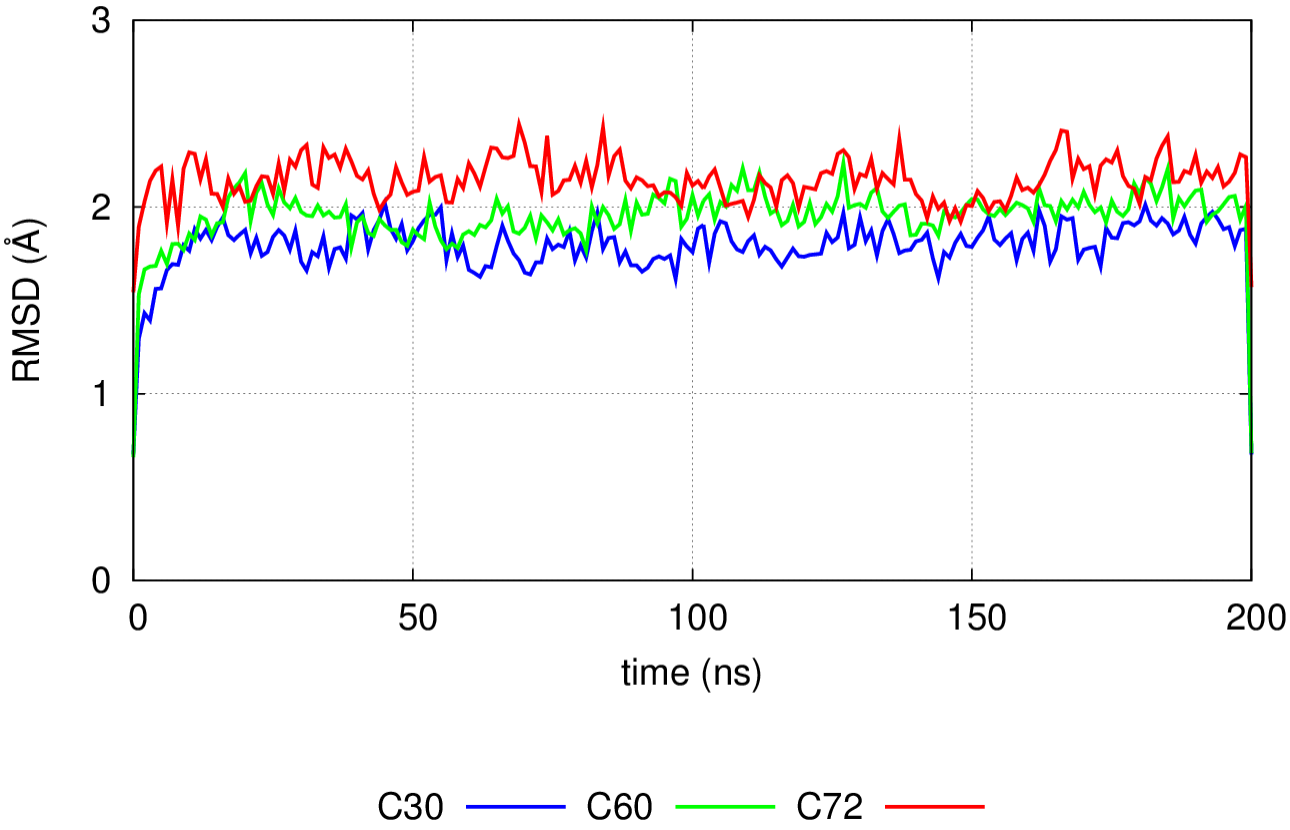

Figure S1. RMSD (relative to the initial TRPV1 core structure) for three representative MD trajectories in the C state at 30°C (C30), 60°C (C60), and 72°C (C72). All three RMSD curves stabilize within 50 ns.

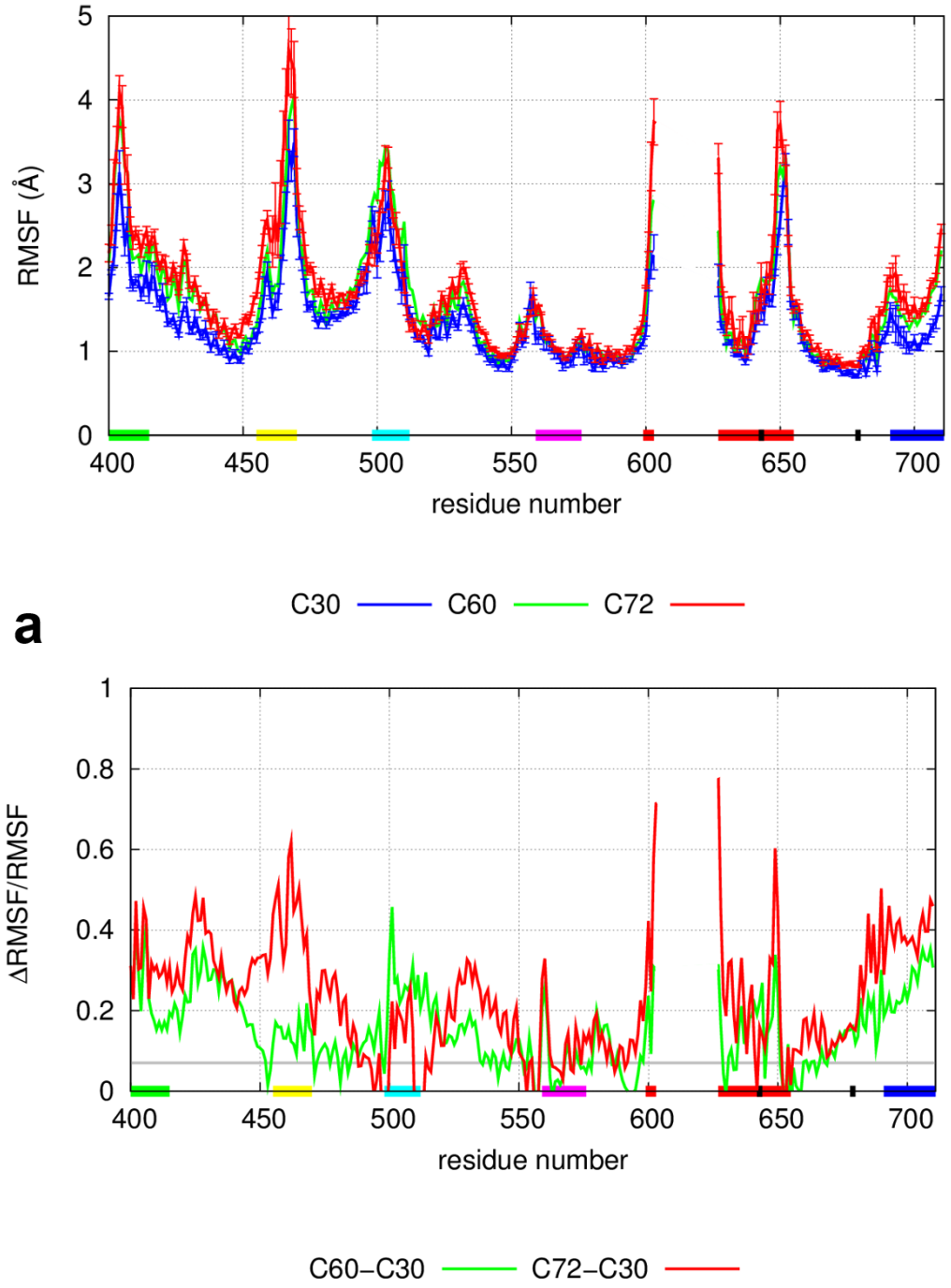

Figure S2 (a) RMSF profiles at 30°C, 60°C, and 72°C which are labeled C30, C60, and C72. (b) The fractional change in RMSF from C30 to C60, and from C30 to C72, where a gray horizontal line corresponds to an increase of RMSF by 7% due to an unspecific thermal effect. Average RMSF for four equivalent residue positions of the TRPV1 tetramer is shown in (a). Error bars for C30 and C72 are also shown in (a). Most of the observed C30-to-C72 RMSF differences are significant with the blue and red error bars not overlapping. The residue positions corresponding to the MPD linker, the S1-S2 linker, the S2-S3 linker, the S4-S5 linker, the outer pore, the TRP helix, and the upper/lower gate are marked by horizontal bars colored in green, yellow, cyan, purple, red, blue, and black, respectively.

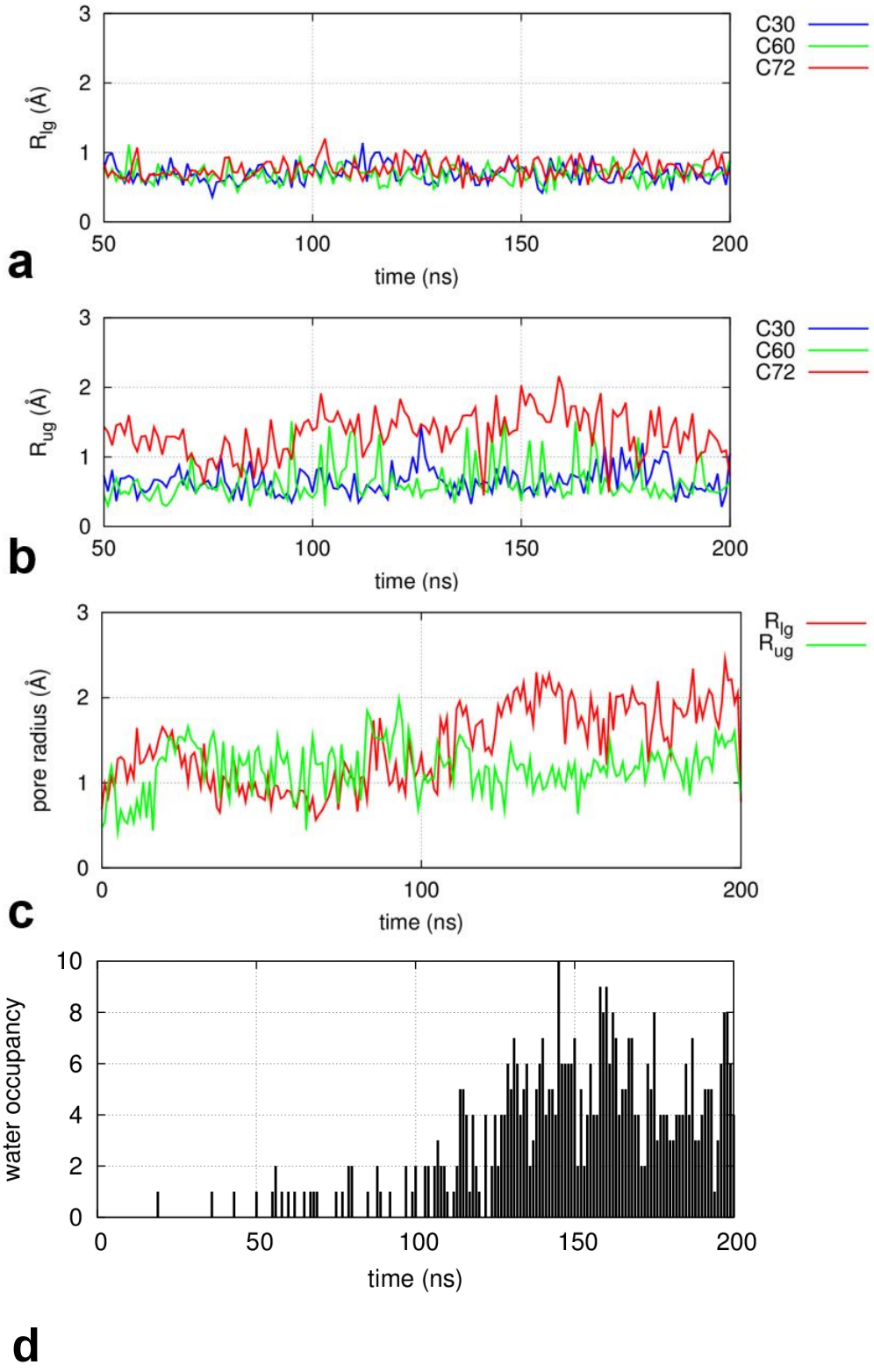

Figure S3 Channel pore analysis for (a) the lower gate, (b) the upper gate in a representative WT trajectory (blue: 30°C, green: 60°C, and red: 72°C), and (c) both lower gate (red) and upper gate (green) in a representative mutant trajectory. (d) shows the water occupancy near the lower gate (within 2 Å of I679 in z coordinate) for the representative mutant trajectory.

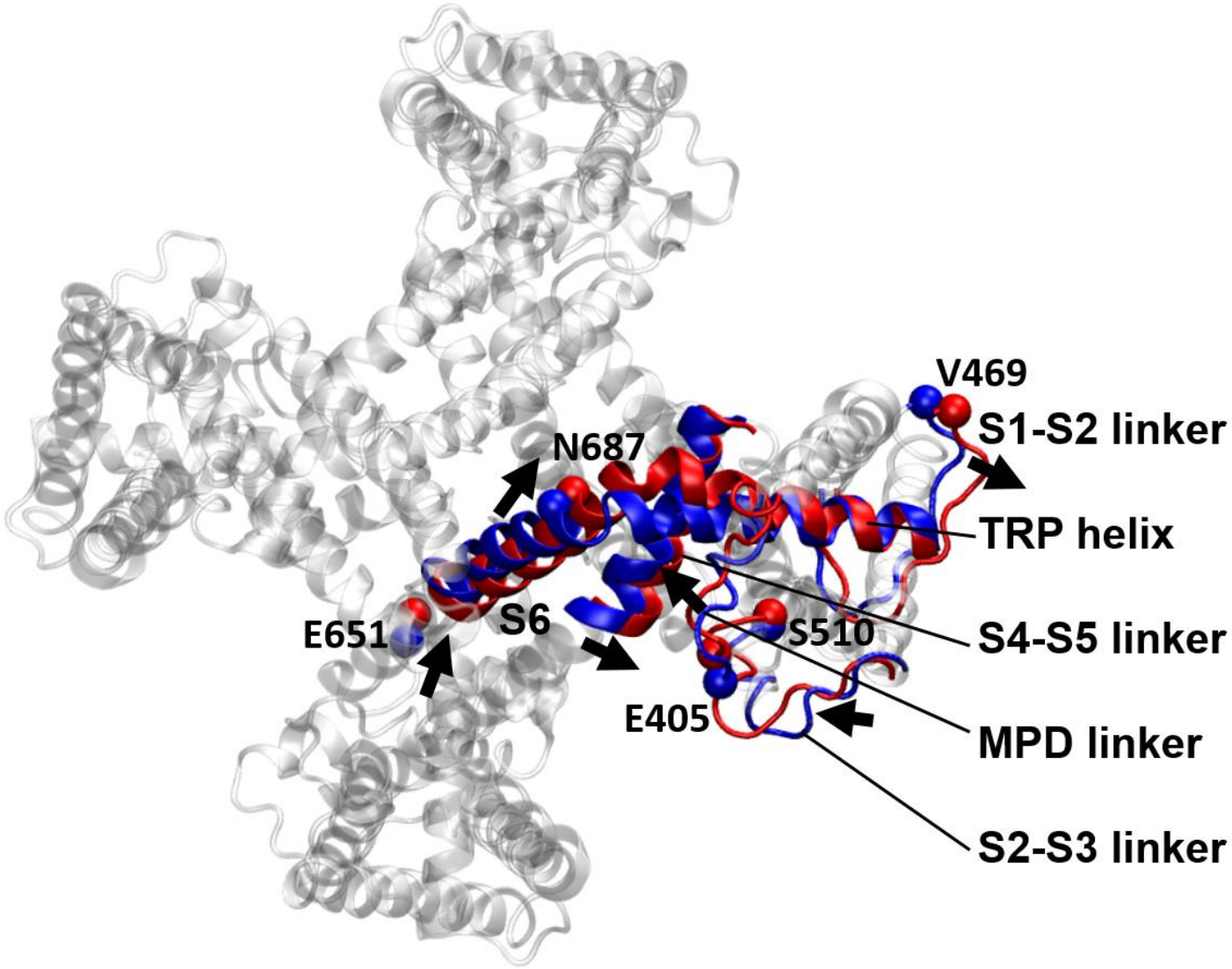

Figure S4. Conformational changes from the closed structure (blue) to the open structure (red) of core TRPV1 (residues 400-710) in the bottom view. For clarity, only the MPD linker, the S1-S2 linker, the S2-S3 linker, the S4-S5 linker, the S6 helix, and the TRP helix of a representative subunit are colored while the rest is in gray. Marker residues for key domains (E405, V469, S510, E651, and N687) are labeled and shown as balls. Domain motions are indicated by black arrows.

Table S1. Gating-associated changes in nonbonded energy within protein, and between protein and membrane/water.

interacting components	electrostatic energy change (kcal/mol)			vdW energy change (kcal/mol)		
protein-water	5978±509[1]	4350±446[2]	5452±429[3]	-98±61[1]		
protein-membrane	-1347±118			-528±20		
intra-protein	-3706±219			157±64	294±30[2]	472±34[3]
intra-ICD	-2253±197			150±38		
intra-TMD	-721±81			-8±33		

[1]. Energy change from 0 ns to 200 ns in the mutant simulations.
[2]. Energy change from 30°C to 60°C in the WT simulations.
[3]. Energy change from 30°C to 72°C in the WT simulations.

Table S2. Top-5% residue positions with the most loss of protein-water HBs.

residue name and id	change in protein-water HB count	
	WT	mutant
from 30°C to 60°C	from 30°C to 72°C	from 0 ns to 200 ns
GLU391 -1.6±0.8	**GLU570** -2.5±0.5	**GLU570** -3.9±0.6
ARG428 -1.3±0.6	**GLU405** -2.0±0.8	ASP388 -3.6±0.7
GLU405 -1.2±0.8	ARG428 -1.5±0.6	GLU692 -2.9±0.7
GLU570 -1.2±0.5	**SER403** -1.5±0.5	**ARG500** -2.6±0.6
THR389 -1.2±0.5	GLU692 -1.4±0.6	ASP427 -2.4±0.7
GLU371 -1.1±0.7	ARG367 -1.3±0.6	GLU536 -2.4±0.6
GLU648 -1.1±0.8	GLU397 -1.2±0.6	GLU371 -2.3±0.8
GLU709 -1.0±0.6	LYS392 -1.1±0.6	ASN467 -2.2±0.6
ASP383 -1.0±0.6	ASN419 -1.1±0.5	**GLU405** -2.2±0.8
ARG363 -0.9±0.6	**ARG575** -1.1±0.5	ARG363 -2.2±0.6
ASN419 -0.9±0.5	GLU391 -1.1±0.8	GLU391 -2.1±0.7
GLU651 -0.9±0.8	ASP427 -1.1±0.7	ALA719 -2.1±0.7
ASN467 -0.9±0.6	GLU709 -1.0±0.6	GLU600 -2.1±0.7
SER403 -0.9±0.5	THR389 -1.0±0.5	**ARG575** -2.1±0.5
ASP427 -0.9±0.7	**SER512** -1.0±0.3	**ARG557** -2.0±0.5

Residues on the MPD linker, the S2-S3 linker, and the S4-S5 linker are in bold font.

Table S3. Heat-activated changes in nonbonded energy between membrane/water and specific TRPV1 domains.

interacting components	electrostatic energy change (kcal/mol)		vdW energy change (kcal/mol)	
	C30-to-C60	C30-to-C72	C30-to-C60	C30-to-C72
membrane &				
MPD	34±37	35±36	-3±4	6±4
S2-S3 linker	63±59	200±55	-5±7	20±7
S4-S5 linker	-112±37	-28±29	-7±5	10±5
outer pore	-28±33	2±32	-8±6	0±6
TRP helix	4±26	32±25	2±3	10±3
water &				
MPD	691±148	554±163	41±21	45±21
S2-S3 linker	81±66	191±67	-3±8	-6±8
S4-S5 linker	202±69	373±61	1±7	-8±7
outer pore	285±126	71±128	-5±13	-18±13
TRP helix	214±85	342±86	6±9	3±9

Movie S1. Permeation of a water molecule (colored by atom type: red for oxygen and white for hydrogen) through the channel pore.

Movie S2. Permeation of a K^+ ion (shown as a blue sphere) through the channel pore.