MOLECULAR DYNAMICS SIMULATIONS UNVEIL THE MECHANISM OF THE SENSITIVITY TO HEAT OF AN ION CHANNEL

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EXECUTIVE SUMMARY

The structure of TRPV1, the ion channel that makes peripheral nerves sensitive to heat and capsaicin, a bioactive component of chili peppers, has been experimentally determined in both the closed and open states. Very little is known about its activation mechanism however. Our research team has discovered a molecular mechanism of activation that involves the rotation of a conserved asparagine in one of the pore lining helices in and out of the pore. This rotation is correlated with the dehydration of four peripheral cavities. In light of this mechanism, we performed bioinformatics analyses of other evolutionarily related ion channels, analyzed newly available structures, and reexamined previously reported water accessibility and mutagenesis experiments. Overall, we have provided several independent lines of evidence that support the newly discovered mechanism.

RESEARCH CHALLENGE

Chronic pain is a diffuse medical condition that affects millions of Americans [1] and is associated with a range of diseases and disorders including diabetic neuropathy, peripheral neuropathy, low back pain, post-therapeutic neuralgia, fibromyalgia, neurological disorders, and arthritis [2,3]. Chronic pain can significantly impact quality of life [4], health, and productivity, with more than $100 billion lost annually in the United States alone [1,5]. In many instances however chronic pain is ineffectively managed [1,2].

Further, currently used therapeutics can have significant side effects including addiction (opioids); limited pain relief (NSAIDs); cognitive impairment or sleepiness (sedatives, antidepressants, anticonvulsants, muscle relaxants); acclimation (opioids); tissue damage (NSAIDs); and gastrointestinal problems (NSAIDS, opioids); as well as limited compliance (capsaicin) [2,5–8]. As a result, a number of efforts are underway in academia, government research institutions, and the pharmaceutical industry to address the unmet medical need of controlling chronic pain.

The transient receptor potential cation vanilloid type 1 (TRPV1) ion channel transduces noxious stimuli into electrical signals and is present in peripheral sensory neurons. Inflammation causes overexpression and sensitization of TRPV1, resulting in increased responsiveness to painful stimuli (allodynia). Modulating the channel activity for pain control is safer for TRPV1 than for other ion channels involved in propagating painful signals. This is because, despite being relatively widely expressed, TRPV1 channels do not play a crucial role in the heart or central nervous system [9]. This makes TRPV1 an extremely promising target for treating chronic pain [2, 9–11].

METHODS & CODES

The structure of the TRPV1 capsaicin-bound (CAP-bound) state was taken from the Protein Data Bank (PDB): the PDB code is 3j5r [12]. We refined the structure and modeled the missing residues using Rosetta software [13]. Four capsaicin molecules were docked following the protocol described in [14]. The protein with the ligands was embedded in a hydrated 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayer and surrounded by 150 mM of NaCl solution. The overall size of the system was approximately 170 × 170 × 160 Å^3; the total number of atoms was approximately 400,000. We generated two MD trajectories with the peripheral cavities (PCs) either empty or hydrated. We used the CHARMM36 force field [15] to describe the protein and the POPC lipids. For capsaicin, we used the parameters derived in [16]. The TIP3P model was used to describe water [17]. An analogous setup was used to simulate the TRPV1 apo state (PDB code 3j5p [18]). We performed the equilibration of the systems (three in total: the CAP-bound state with empty and hydrated PCs, and the apo state) using NAMD 2.10 software [19] in several steps. Simulations were performed at constant temperature and pressure (1 atm) using the Langevin piston approach. For the vdW interactions, we used a cutoff of 11 Å with a switching function between 8 and 11 Å. We calculated the long-range component of electrostatic interactions using the Particle Mesh Ewald approach [20] with a cutoff of 11 Å for the short-range component. The equations of motion were integrated using a multiple timestep algorithm, with a timestep of 2 femtoseconds (fs) and long-range interactions calculated every other step.

We performed metadynamics simulations using the preliminary unbiased trajectories to estimate an upper bound for the free energy barrier and the diffusion constant along the biased collective variable. These were used to obtain an *a priori* estimate of the error on the reconstructed free energy profile using the expressions reported in [21], which relate the error to the width, height, and deposition rate of the hills. Metadynamics simulations were performed using the collective variable module implemented in NAMD 2.10 [22] at three temperatures: 300K, 280K, and 340K.

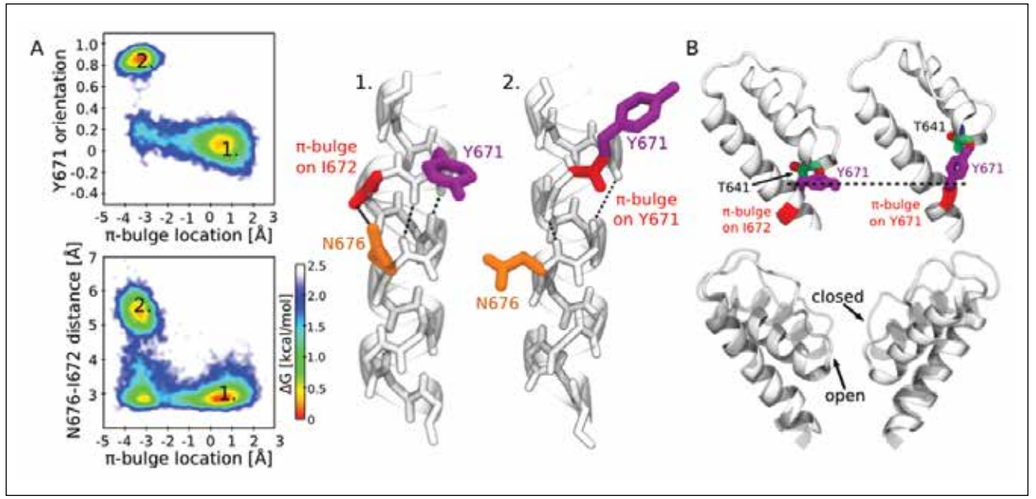

Figure 1: (A) Coupling between N676 and Y671. Top left panel: Y671 orientation and the π-bulge position. Only two conformations are observed: open state (1) and closed state (2). The bottom left panel shows the distance between N676 carboxamide carbon and I672 carbonyl oxygen and the π-bulge position. (B) A change of Y671 orientation results in a displacement of the pore helix.

RESULTS & IMPACT

We found that TRPV1 activation involves the rotation of an evolutionarily conserved amino acid located in the middle of the pore lining helix S6 (N676), which projects its side chain toward either the pore or the S4–S5 domain (Fig. 1). Hydration of the pore and thus conduction of ions is only possible in the former case. We found that the conformational switch of N676 is correlated with a wet-to-dry transition in four so-far-unreported peripheral cavities. The presence of the peripheral cavities reconciled seemingly contradictory observations about the accessibility of S6 residues to solvent. We tested our model by introducing mutations in residues lining these cavities in S6 [24,25]. We were thus able to confirm our microscopic mechanism of activation [25].

Given their crucial relevance for activation, these peripheral cavities offer a completely new opportunity to modulate the activation of TRPV1 through small molecule binding. The observation that several drugs bind to the corresponding region in structurally homologous channels such as TRPML, TRPA1, and NaChBac [26–28] lends confidence to the possible druggability of these pockets.

Many attempts at developing TRPV1 antagonists have failed due to side effects and lack of efficacy. The six most advanced candidates in clinical trials have only completed Phase II since 2011; some of these have not progressed despite the passage of considerable time, likely because their development could not take advantage of the newly available structural information. By providing a microscopic picture of the activation mechanism, our study will allow researchers to expand the chemical space and select optimal compounds.

WHY BLUE WATERS

We investigated a system of approximately 400,000 atoms using molecular dynamics. The time scales involved in the activation process of TRPV1 dictated trajectory lengths on the microsecond range. This extensive simulation was possible thanks to a massively parallel calculation enabled by the computational capabilities of Blue Waters.