**SUPPLEMENTARY INFORMATION**

# Atomistic mechanisms of human TRPA1 activation by electrophile irritants through molecular dynamics simulation and mutual information analysis

Matthew Habgood[1,2*], David Seiferth, Afroditi-Maria Zaki[1], Irfan Alibay[1], and Philip C. Biggin[1*]

[1]Department of Biochemistry, University of Oxford, South Parks Road, Oxford. OX1 3QU. UK.

[2]AWE Aldermaston, Reading, Berkshire, RG7 4PR. UK.

**Fig S1. Individual RMSD time series of the backbone atoms for each repetition.** RMSDs are shown for simulations based on experimental structures 6PQQ (**A-C**), 6PQP (**D-F**) and 6V9X (**G-I**).

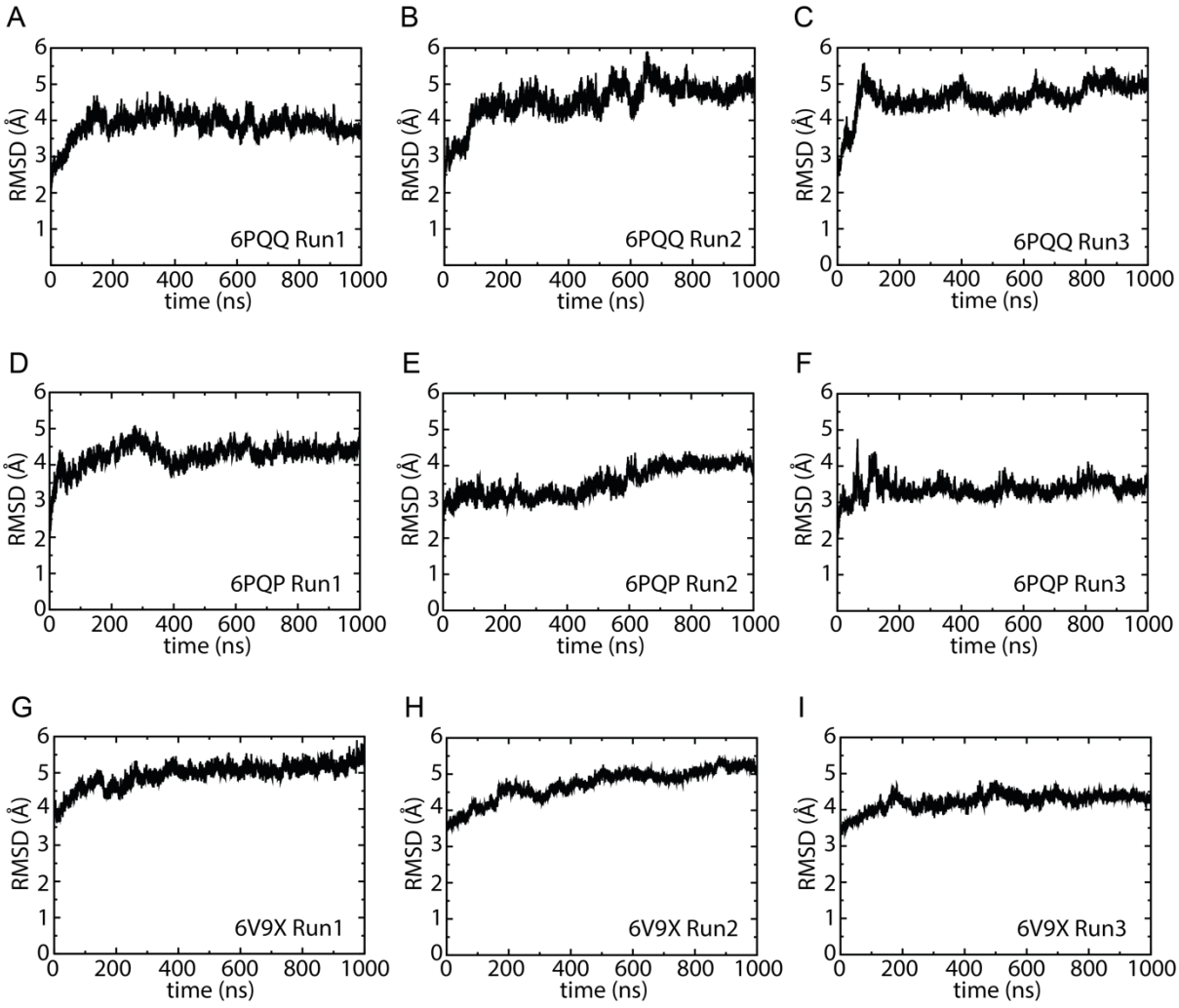


**Fig S2. Pore radius profiles.** Pore radius (computed for the transmembrane region only) as a function of the *z* coordinate within the simulation box are shown for simulations based on experimental structures for (**A-C**) 6PQQ, (**D-F**) 6PQP and (**G-I**) 6V9X. V961, which is considered as the main gating residue is located at z = -24 Å.

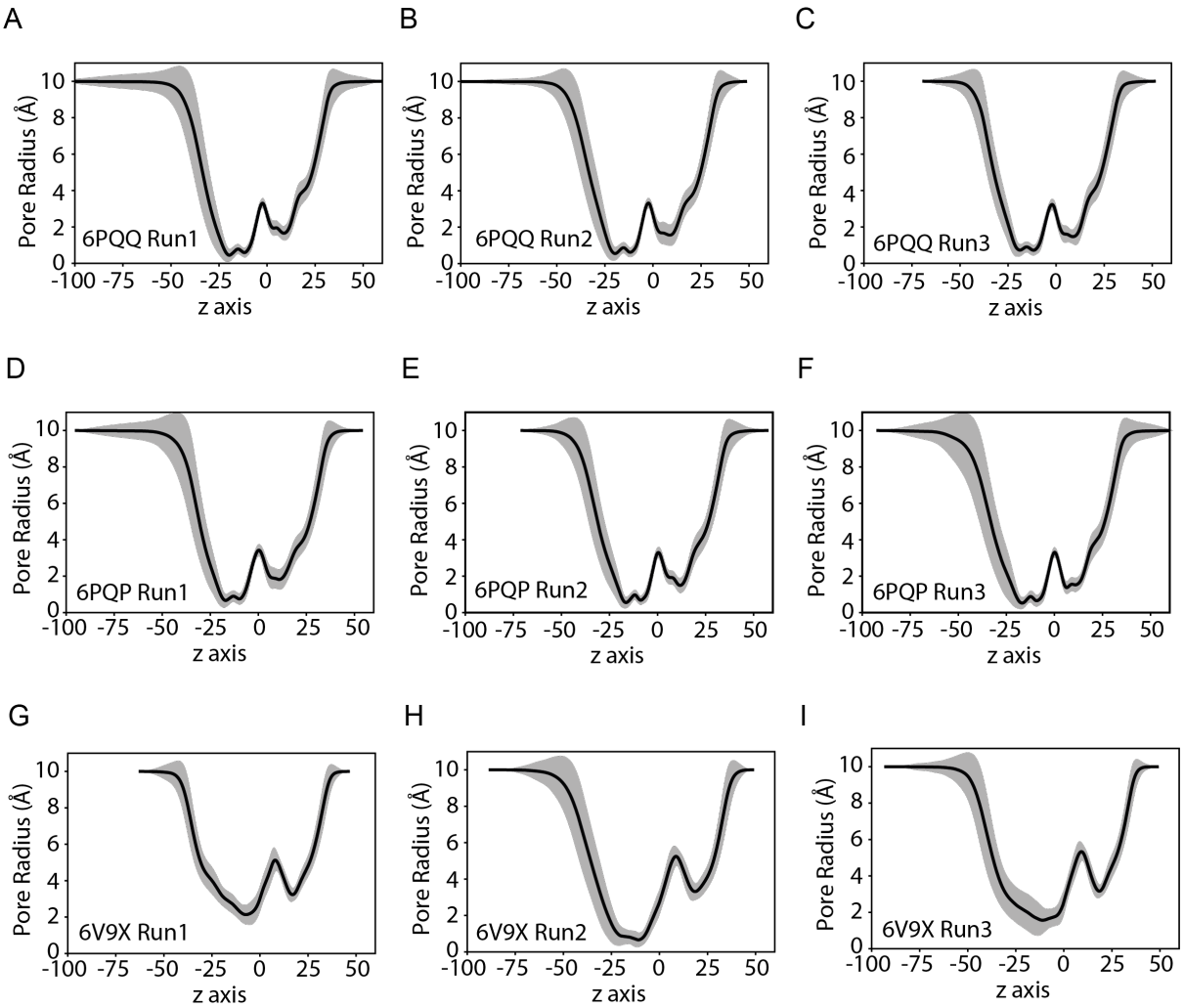


**Fig S3. Pore radius profiles for 6V9X in various alternative conditions.** Pore radius (computed for the transmembrane region only) as a function of the *z* coordinate within the simulation box are shown for simulations for (**A-C**) 6V9X with the ligand deleted, (**D-F**) 6V9X with iodoacetamide covalently bound to Cys665 and (**G-I**) 6V9X with benzyl isothiocyante (BITC) covalently bound at Cys621. V961, which is considered as the main gating residue is located at z = -24 Å.

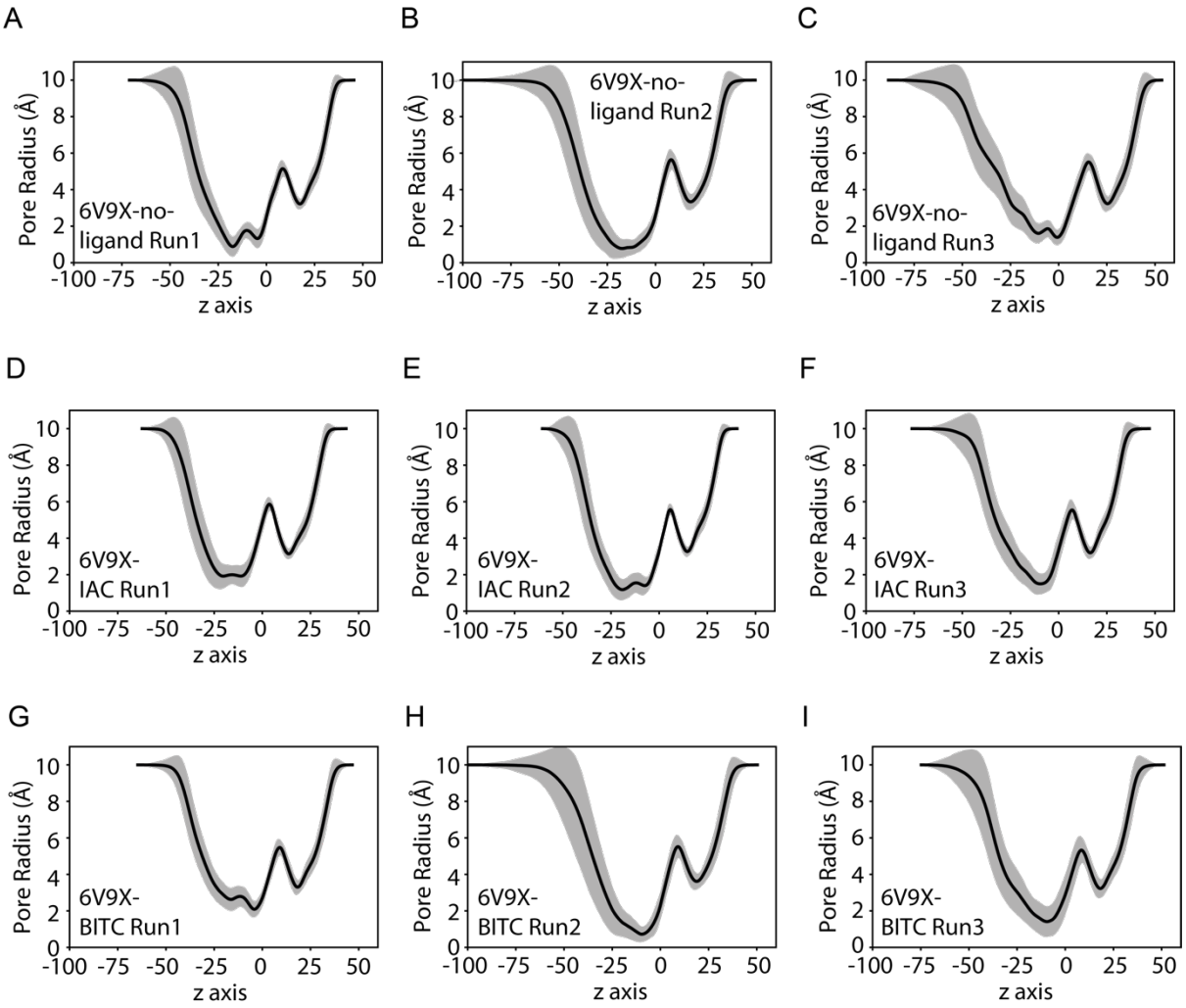


**Fig S4. Pocket width time series.** The width of the pocket as indicated by the $C_\alpha$-$C_\alpha$ distance between residues Lys610 (lower lip) and Leu 667 (upper lip) for (**A-C**) 6PQQ, (**D-F**) 6PQP and (**G-I**) 6V9X simulations. Corresponding distances in 6PQQ (pore closed, pocket closed) and 6V9X (pore open, pocket open) are 9.4 Å and 15.5 Å, respectively.

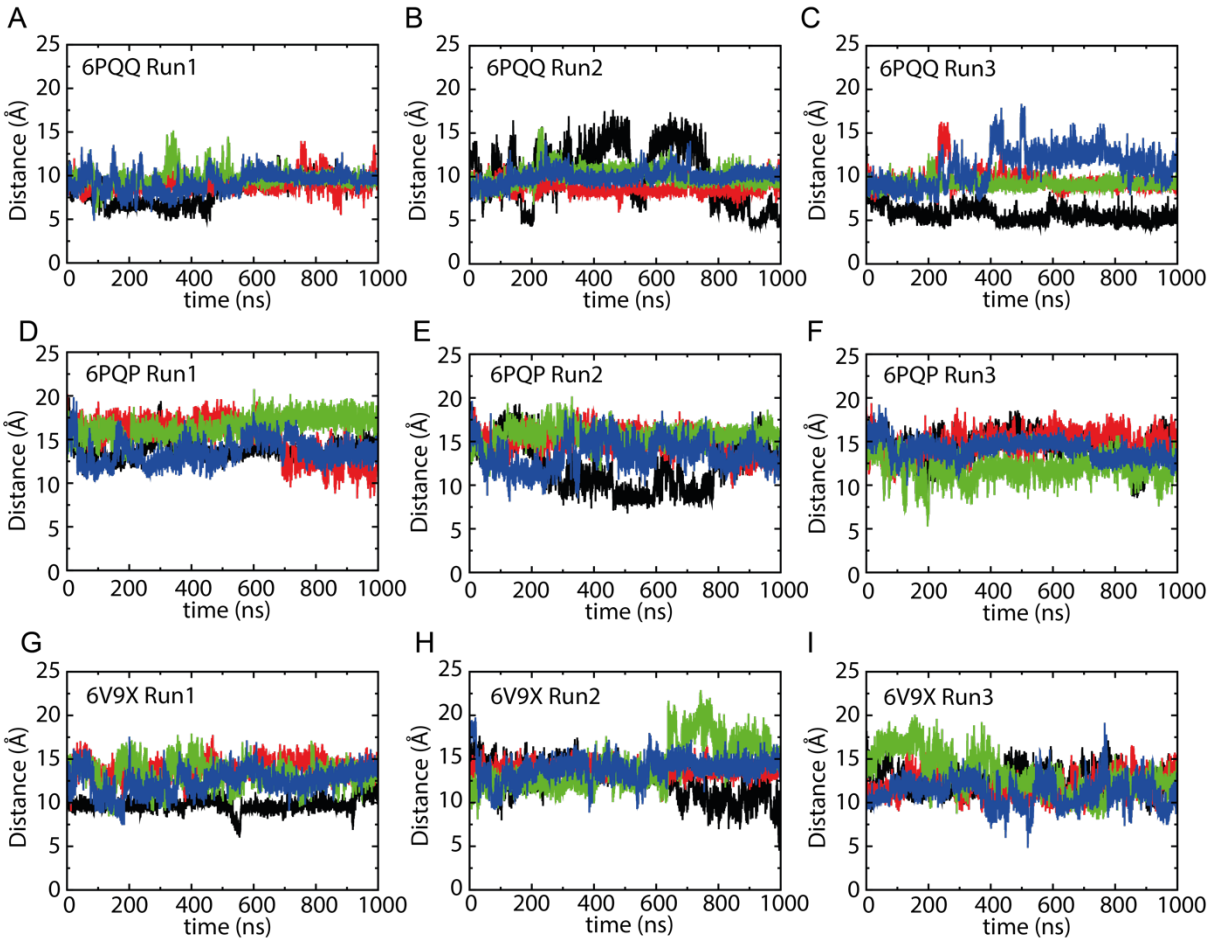
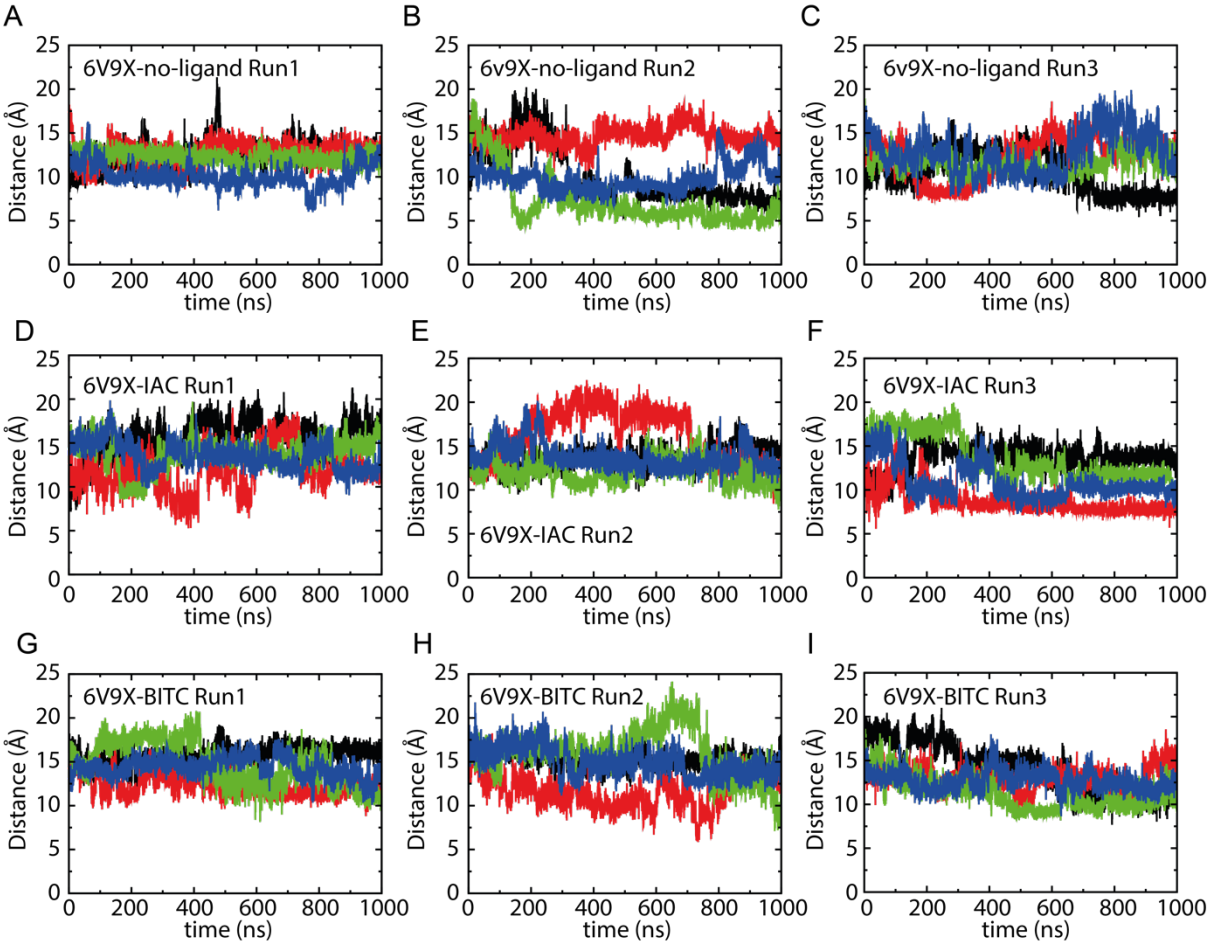


**Fig S5. Pocket width time series for 6V9X in various alternative conditions**. The width of the pocket as indicated by the Cα-Cα distance between residues Lys610 (lower lip) and Leu 667 (upper lip) for (**A-C**) 6V9X with the ligand deleted, (**D-F**) 6V9X with iodoacetamide covalently bound to Cys665 and (**G-I**) 6V9X with benzyl isothiocyante (BITC) covalently bound at Cys621. Corresponding distances in 6PQQ (pore closed, pocket closed) and 6V9X (pore open, pocket open) are 9.4 Å and 15.5 Å, respectively.



**Text S6. Mutual information data**

Pairwise mutual information was calculated between the residues of a set of chain segments in the region between the cysteine 621 ligand binding pocket, and the ion pore. As shown in the main paper, Figure 1A, these segments are: the ligand binding pocket lid (H4) (Gln664-Thr684); the loop above the pocket lid (H7), TRP-like domain and transmembrane domain helix (part of S6) (Val961-Lys1001) ; the VSLD helix threaded through loop above pocket lid (S1) (Tyr706-Val737); and the VSLD helix (S4) and connected TMD helix immediately adjoining Val961 (S5) (Gln831-Val861).

For analysis purposes, these were further split down into seven sub-segments:

Segment 1: the binding pocket lid (H4) [Gln664 – Thr 684]

Segment 2: the loop above the binding pocket lid (H7) [His983 – Lys1001]

Segment 3: the section of the outer upright in the VSLD adjacent to segment 2 (S1) [Tyr706 – Tyr714]

Segment 4: the upper part of the outer upright in the VSLD (S1) [His719 – Val737]

Segment 5: the inner upright in the VSLD (S4) [Gln831 – Phe 853]

Segment 6: the loop in the transmembrane domain, connected to S4, adjacent to the TRP domain and pore-bordering helix (S5) [Glu854 – Val861]

Segment 7: the TRP domain and pore-adjacent helix (S6) [Val961 - Gln979]