
α_hENaC(6WTH)
β_hENaC(6WTH)
γ_hENaC(6WTH)
A_cASIC1(4NTX)
B_cASIC1(4NTX)
C_cASIC1(4NTX)
α_xENaC(model)
β_xENaC(model)
γ_xENaC(model)

Figure S1. Sequence alignment used in modeling xENaC. The templates were hENaC according to 6WTH structure [13] and cASIC1 according to 4NTX structure [14]. The alignment was generated using Modeller v10.1 [34].

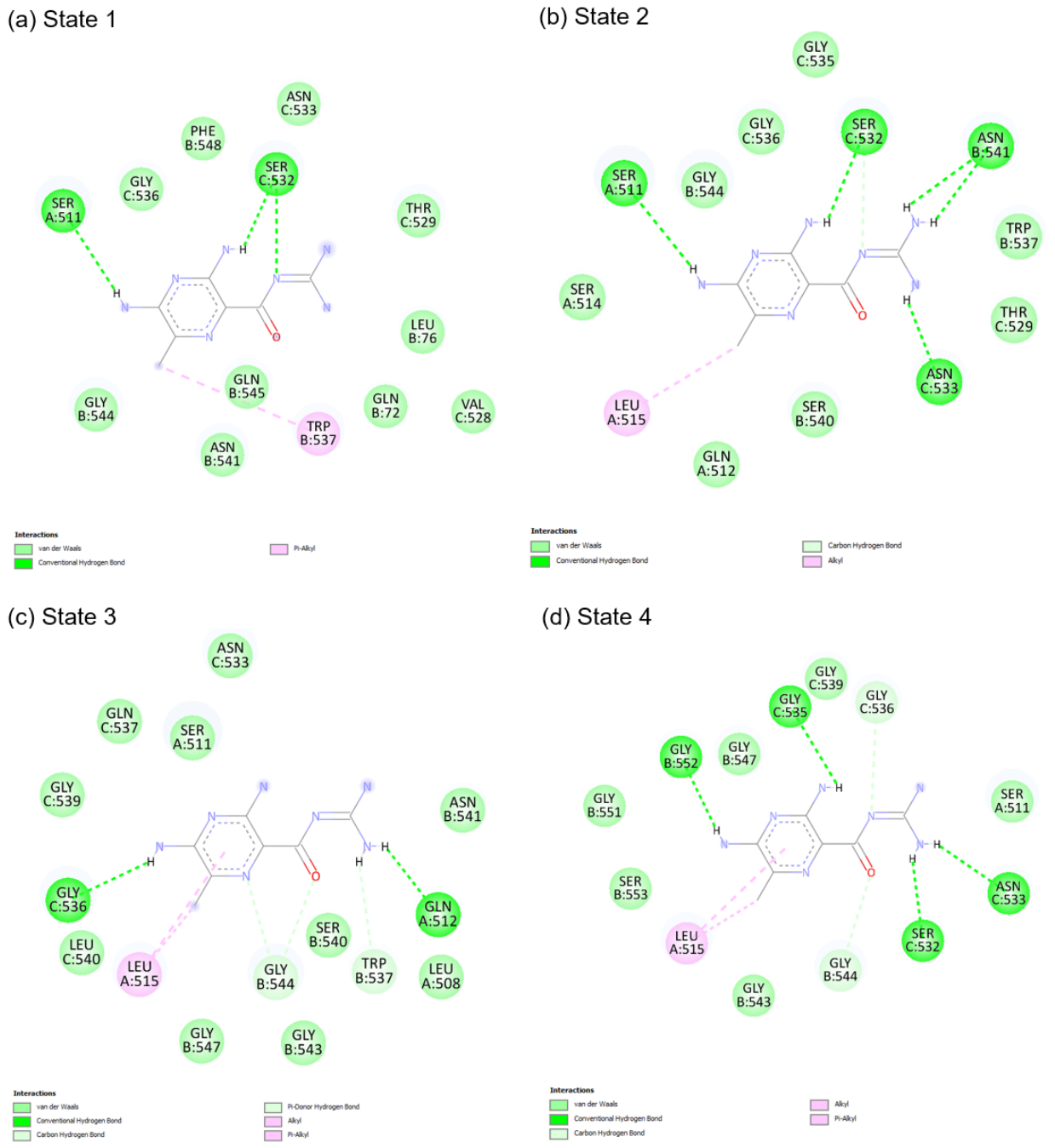

Figure S2. 2D interaction maps of amiloride with the representative structures in the four states: (a) state 1 – after ~1.45 ns of equilibration dynamics (-0.5 ns to the production dynamics), (b) state 2 – after ~2 ns of production dynamics, (c) state 3 – after ~10 ns of production dynamics and (d) state 4 – after ~16 ns of production dynamics. The interactions are represented as follows: dark green for conventional hydrogen bonds (the hydrogen bonds are green dashed lines), light green for van der Waals interactions, very light green for carbon - hydrogen bonds or pi-donor hydrogen bonds, pink for alkyl or pi-alkyl interactions, orange for pi-carbon interactions and red for unfavorable interactions.

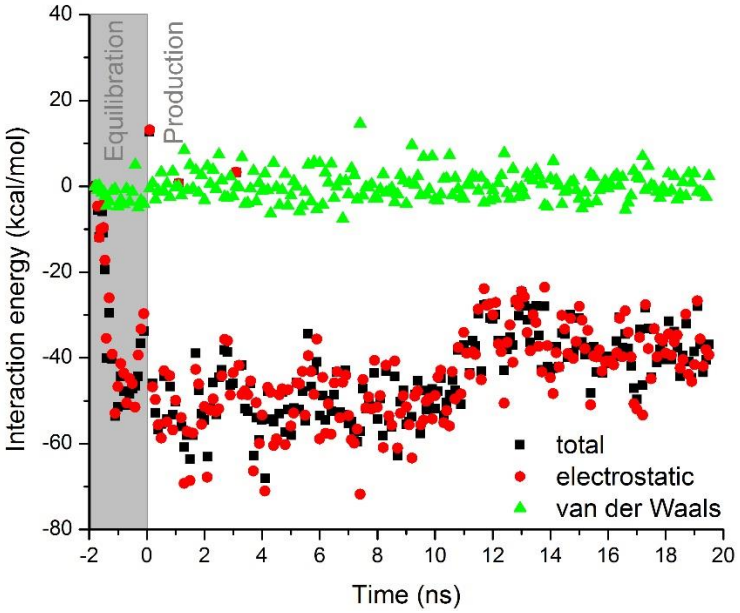

Figure S3. Interaction energies of amiloride with water molecules. Black is used for total interaction energy, red for the electrostatic interaction energy and green for van der Waals interaction energy. The grey background was used to highlight values obtained for the equilibration dynamics and white background is used for values associated with the production dynamics.

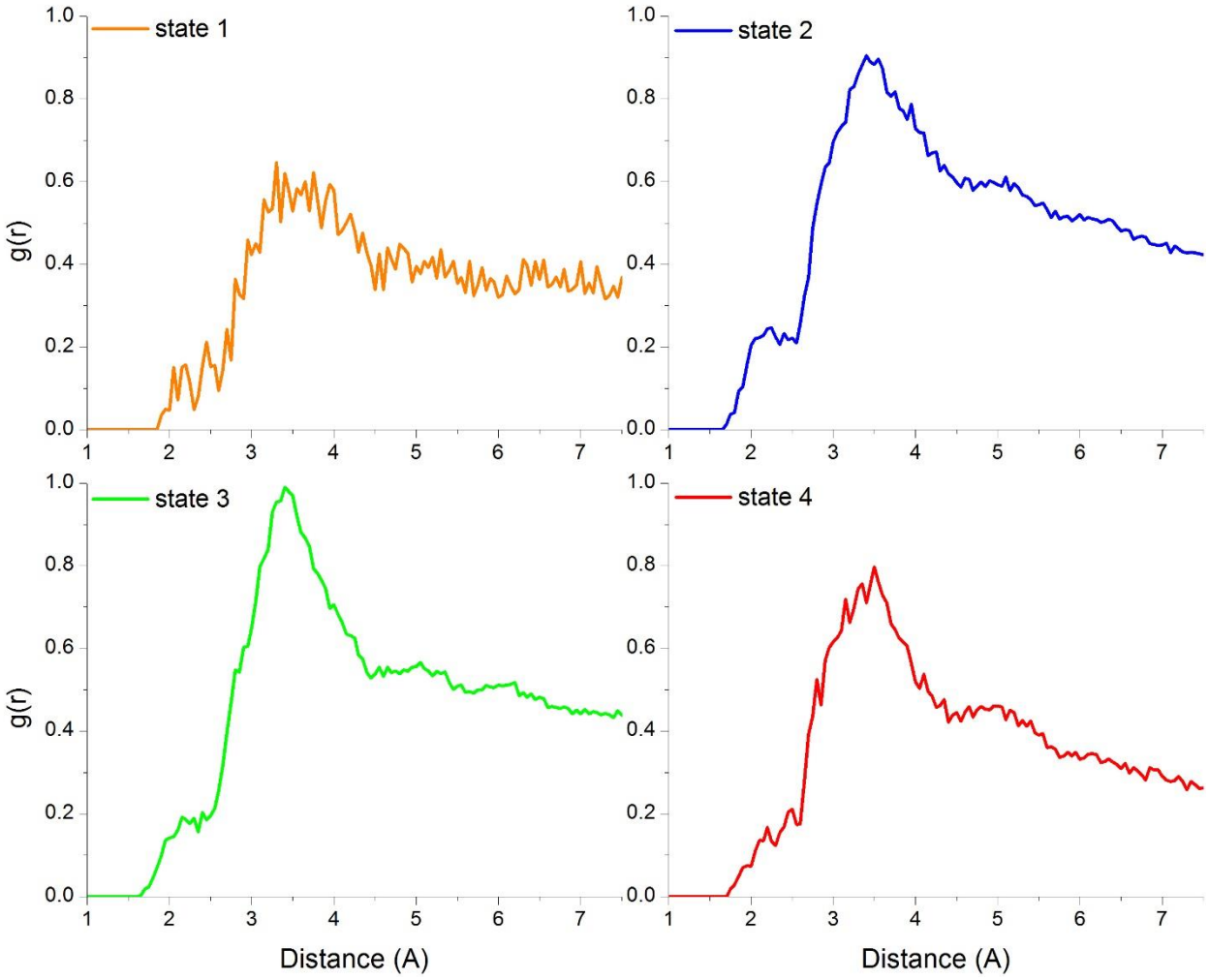

Figure S4. Radial distribution functions (g(r)) of water oxygen atoms around amiloride. The functions were calculated on MD trajectories corresponding to the four states of amiloride, namely state 1 – the last 1.5 ns of equilibration dynamics, state 2 – the range 2 – 6 ns of production MD, state 3 – the range 6-10 ns of production MD, state 4 – the range 12 – 20 ns of production MD. Calculations were performed considering the water molecules on a distance of 7.5 Å around amiloride.

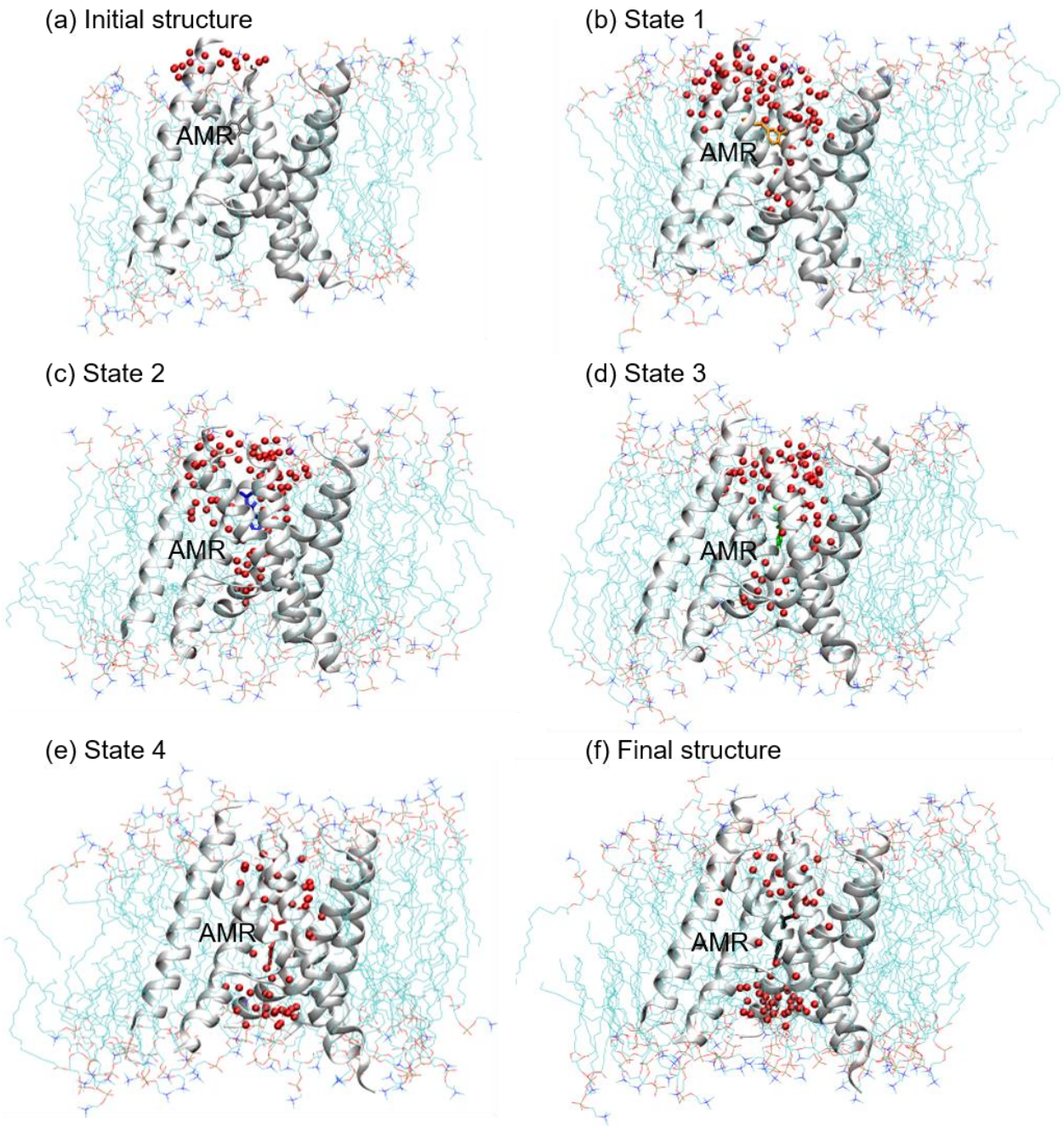

Figure S5. Snapshots of xENaC TM region with bound amiloride. Amiloride is found in the positions associated with the initial structure (a), the four states accessed during the dynamics: after ~1.45 ns of equilibration (b – state 1), after ~2 ns of production dynamics (c – state 2), after ~10 ns of production dynamics (d – state 3), after ~16 ns of production dynamics (e – state 4) and the final structure, after 20 ns of production dynamics (f). The protein is represented in white cartoons. Amiloride is represented as licorice and colored according to its position: grey in (a), orange in (b), blue in (c), green in (d), red in (e) and black in (f). Amiloride coloring scheme is consistent with that in Figure 4 (c). The oxygen atoms from

the water molecules within 10 Å of amiloride are represented as red spheres and lipids within 5 Å of the protein are represented as lines colored according to atom types.