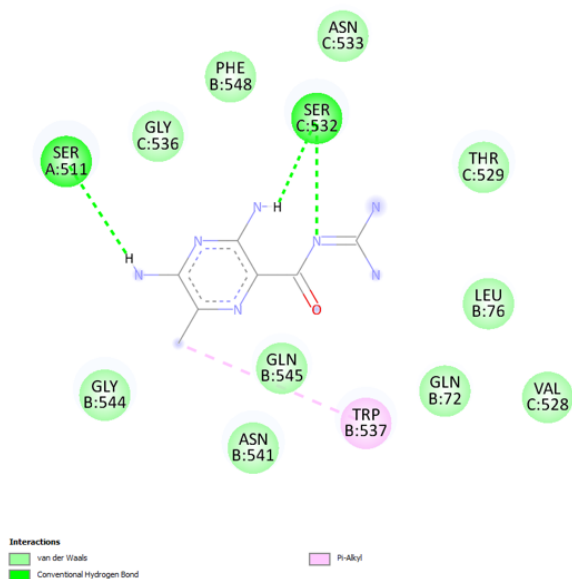


# Supplementary material

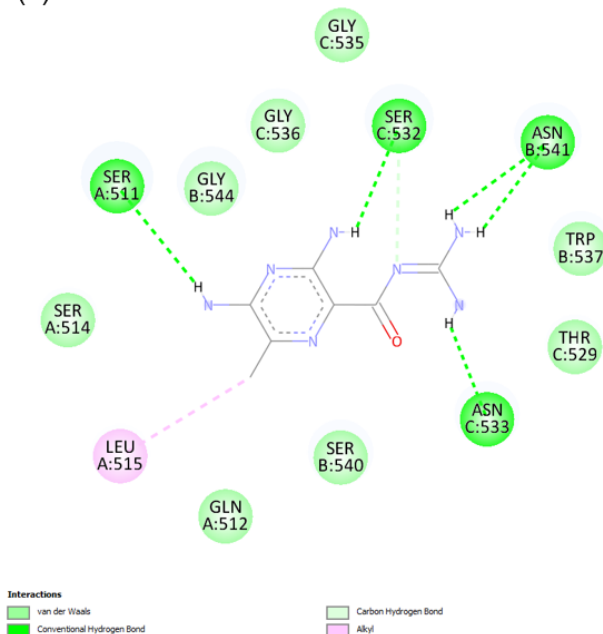
|                       |  |     |
|-----------------------|--|-----|
| <i>α_hENaC(6WTH)</i>  | 114 -----VSLNIN-LNSDKLVFPAVTICTLNPYRYPEIKEELEELDRITEQTLFDLY 162                            |     |
| <i>β_hENaC(6WTH)</i>  | 78 -----EVSVSLV-VGFKTMDFAVTICNASPFKYSKI-KHLLKDLDELMEAVLERI 126                             |     |
| <i>γ_hENaC(6WTH)</i>  | 80 -----TVSVSIKVHFRKLDFAVTICNINPYKYSTRHLLADEQETREALKSLY 129                                |     |
| <i>A_cASIC1(4NTX)</i> | 45 VVWALCFMGS LALLALVCTNR IQYYFLYPHVTKLDEVAATRLTFPAVTFCNLNEFRF-----SRVTKNDLYH-- 111        |     |
| <i>B_cASIC1(4NTX)</i> | 45 VVWALCFMGS LALLALVCTNR IQYYFLYPHVTKLDEVAATRLTFPAVTFCNLNEFRF SRVTKNDLYHAGELLALLNNRY 123  |     |
| <i>C_cASIC1(4NTX)</i> | 45 VVWALCFMGS LALLALVCTNR IQYYFLYPHVTKLDEVAATRLTFPAVTFCNLNEFRF SRVTKNDLYHAGELLALLNNRY 123  |     |
| <i>α_xENaC(model)</i> | 49 AFWLVLFLVTFGLMYWQFGLLFGQYFSPVSVINLN-VNSDKLPFAVTVCTLNPPYRYKAIQNDLQELDKETQRTLYELY 126     |     |
| <i>β_xENaC(model)</i> | 56 VMVFILTLVFAGLVFWQAGVLILTYLSYGVSVSLV-IGFKTMEFPAVTLNANPFPKYSRV-KPLLKELDELVATALDR 132      |     |
| <i>γ_xENaC(model)</i> | 55 NIWISLTLCVAVAVI FWQCALLLMSYY--SVSASI TVTFQKLYVPAVTICNLNPYSYSKVKVDRLAALEKETSTQTLKNIY 130 |     |
| <i>α_hENaC(6WTH)</i>  | 163 KYSS-----LPHPLQRL-----KIGFQLCNQNKSDCFYQTYSSGV 198                                      |     |
| <i>β_hENaC(6WTH)</i>  | 127 -----LAPELNLNFSIVNHTPLVLIDERNPHHPMVLDLFASEKICNAHG-----KMA 174                          |     |
| <i>γ_hENaC(6WTH)</i>  | 130 GFPEPR-----FSHRIPLLIFDQV-----VGFLQC 154  |     |
| <i>A_cASIC1(4NTX)</i> | 112 -----AGELLALL-----NNRYEIPDTQTAD-----EKQL 136   |     |
| <i>B_cASIC1(4NTX)</i> | 124 -----EIPDTQTADEKQLEILQD-----ANFR-- 146   |     |
| <i>C_cASIC1(4NTX)</i> | 124 EIPDTQ-----TADEKQLEILQDK----- 146  |     |
| <i>α_xENaC(model)</i> | 127 KYNS TGVQQWI PNNQRVKRDRAGLPYLLELPPGSETHRVSRVIEEELQVKRREWNIGFKLCNETGGDCFYQTYTSGV 205    |     |
| <i>β_xENaC(model)</i> | 133 QFSSQNGNTFTHNNQTRQNVITDLPALVNHILPLVVIDETDPRNP IHNIFDNNNAVYSKNSSIRNSSDQTSYSQRKVA 211    |     |
| <i>γ_xENaC(model)</i> | 131 GFT EPLIRSKRDVG VNVENSTEDIFLKQIPLYRLSEVKSQQLVSDLTKKRTRMSAKVIHRDAESVQDPGNMVGFKLC 209    |     |
| <i>α_hENaC(6WTH)</i>  | 199 DAVREWYRFHYINILSRLPETLPSE----EDTLGNFIACFRNQVSCNQANYSHFHHPMYGNCTYFN--DKNNSNLW 270       |     |
| <i>β_hENaC(6WTH)</i>  | 175 MRLCSLNR TQCTFRNFTSATQALTEWYILQATNIFAQVPPQELVEMSYPGQMILACLFGAEPNRYNFTSIFYPHYGN 253     |     |
| <i>γ_hENaC(6WTH)</i>  | 155 -SNDTSDCATYTFSSGINAIQEWKLYHMYNIMAQVPLEKKINMSYSAEELLVTCFFDGVSCDARNFTLFHHPMHGNCTY 232    |     |
| <i>A_cASIC1(4NTX)</i> | 137 EILQDKANFRNFK-----PKPFNMLEFYDRAGHDIREMLLSCFFRGEQCSPEDFKVVVTR-YGKCYTFNAGQDGKPR 208      |     |
| <i>B_cASIC1(4NTX)</i> | 142 -----KANFRNFKPKPFNMLEFY-----DRAGHDIREMLLSCFFRGEQCSPEDFKVVVTR-YGKCY 194                 |     |
| <i>C_cASIC1(4NTX)</i> | 147 -----NFKPKPFNMLEFY-----RAGHDIREMLLSCFFRGEQCSPEDFKVVVTR-YGKCY 197                       |     |
| <i>α_xENaC(model)</i> | 206 DAIREWYRFHYINILARVQE-AAID-----GEQLENFIACFRNEESCTKANYSSFHHAIFYGNCTYFNQNSDQSNLW 278      |     |
| <i>β_xENaC(model)</i> | 212 MKLCTNNNTQCVYRNFTSGVQALREWYLLQLSSIFSNVPLSGRIDMGFAEDLILTCFLGGQPCSRYNFTHIYDADYGN 290     |     |
| <i>γ_xENaC(model)</i> | 210 DPKNSSDCTIFTFSSGVNAIQEWYRLHYTNILAKISMEDKIAMGYKADELIVTCFFDGLSCDARNFTLFHHPLYGNCTY 288    |     |
| <i>α_hENaC(6WTH)</i>  | 271 MSSMPGINNGLSLMLRAEQNDFIPLLSV-----TGARVMVHGQDEPAFMDGGFNLRPGVETSI SMRKETLDR LGGD 343     |     |
| <i>β_hENaC(6WTH)</i>  | 254 CYIFNMGMT-EKALPSANPGTEFLKLLIDIGQEDYVPFLAST-----AGVRLMLHEQRSYPFIRDEGIYAMSGTETS 325      |     |
| <i>γ_hENaC(6WTH)</i>  | 233 FNNRENET-ILSTSMGGSEYGLQVILYINEEYNPFLVSS-----TGAKVIIHRQDEYFPVEDVGTETAMVTSIGM 304        |     |
| <i>A_cASIC1(4NTX)</i> | 209 ITMKGGTGNGLEIMLDIQQDEYLPWAGETDETSFEAGIKVQIHSQDEPPLIDQLGFGVAPGQFTFVSCQEQRILYLP 287      |     |
| <i>B_cASIC1(4NTX)</i> | 195 CYTFNAGQDGKPRILITMKGGTGNGLEIMLDIQQDEYLPWAGETDETSFEAGIKVQIHSQDEPPLIDQLGFGVAPGQFT 273    |     |
| <i>C_cASIC1(4NTX)</i> | 198 FNAGQDGKPRILITMKGGTGNGLEIMLDIQQDEYLPWAGETDETSFEAGIKVQIHSQDEPPLIDQLGFGVAPGQFTFVSC 276   |     |
| <i>α_xENaC(model)</i> | 279 SSSMPGIKNGLTLVLRTEQHDYIPLLSV-----AGARVLVHGKKEPAFMDNGFNIPPGMETSIGMKKETINRLGGK 351       |     |
| <i>β_xENaC(model)</i> | 291 CYIFNMGQEGENTMSSANPGADFLGLKLVDIEQGEYLPFLQTT-----AAARLILHQQRSFPFVKDLGIYAMPGTETS 363     |     |
| <i>γ_xENaC(model)</i> | 289 FNSAERGN-LLVSSMGAEYGLKVVLVIDEYNPYLSTA-----AGAKILVHDQDEYPIEYLGTELETATETSIGM 360         |     |
| <i>α_hENaC(6WTH)</i>  | 344 YGDC TKNGSDVPVENLYPSKYTQQVCIHSCFQESMIKECGCAYIFYPRPQNVEYCDYRKHSSWGYYKLQVDFSSDHL 422     |     |
| <i>β_hENaC(6WTH)</i>  | 326 IGVLDKLRMGEPYSPCTVNGSEVPVQNFYSDYNTTYSIQACLRSCFQDHMIRNCNCGHYLYPLPRGEKYCNRRDPD 404       |     |
| <i>γ_hENaC(6WTH)</i>  | 305 HLTESFKLSEPYSCQTEDGSDVPIRNIYNAAYSLQICLHSCFQTKMVEKCGCAQYSQPLPPAANYCNYQQHPNMYCY 383      |     |
| <i>A_cASIC1(4NTX)</i> | 288 AGDCKATTGE-----FYDTYSITACRIDCETRYLVENCNRMVH--MPGDAPYCTPEQYKE--CADPALDFLVEKD 354        |     |
| <i>B_cASIC1(4NTX)</i> | 274 VSCQEQRILYLPWPWGDCKATT-----GEFYDTYSITACRIDCETRYLVENCNCR--MVHMPGDAPYCTPEQYKE 341        |     |
| <i>C_cASIC1(4NTX)</i> | 277 QEQRILYLPWPWGDCKATTGE-----FYDTYSITACRIDCETRYLVENCNRMVH--MPGDAPYCTPEQYKE--CAD 344       |     |
| <i>α_xENaC(model)</i> | 352 YSDCEDGSDVDVKNLQSEYTEQVCVRSCFQAAMVARCGCGYAFYPLSPGDQYCDYNKHKHSGHCYYKLIEFTSNKL 430       |     |
| <i>β_xENaC(model)</i> | 364 ISVLVDQLEHMEAPYSSCTVNGSDIPVQNLAEFNSSYSIQSLRSCYQEEVMYKTKCAHYQYPLNGSEYCTNMKHPD 442       |     |
| <i>γ_xENaC(model)</i> | 361 QLTESAKLSDPYSDCTMDGRDVSVENLYNKKYTLQICLNSCFQREMRVSCGCAHYDQPLNGAKYCNYYEYPSWIYCYF 439     |     |
| <i>α_hENaC(6WTH)</i>  | 423 GCF TKCRKPCS VTSYQLSAGYSRWPSVTSQEWFMQLSRQNNYTVNNKRNQVAKVNIFFKELNYKTNSSEPS----- 494     |     |
| <i>β_hENaC(6WTH)</i>  | 405 NAHCYSDLQMSVAQRETCIGMCKESCNDTQYKMTISMADWPSEASEDWIFHVLSQERDQ----TLSRKGIVKLNIYFQE 479    |     |
| <i>γ_hENaC(6WTH)</i>  | 384 QLHRAVQEELGCQSVCKEACSFKEWLTTLTSLAQWPSVVEKWLPLVLTWDQGRQVNNKLNKTDLAKLLIFYKDLNQRS 462     |     |
| <i>A_cASIC1(4NTX)</i> | 355 NEYCVCEMPCNVTRYGKELSMVKIPSKASAKY----LAKKYNKSEQYIGENILVLDIFFEALNYETIEQKKAYEVAGLL 429    |     |
| <i>B_cASIC1(4NTX)</i> | 342 ---CADPALDFLVEKDNEYCVCEMPCNVTRYGKELSMVKIPSKASAKYLAK--KYNKSE----YIGENILVLDIFFEA 411     |     |
| <i>C_cASIC1(4NTX)</i> | 345 PALDFLVEKDNEY-CVCEMPCNVTRYGKELSMVKIPSKASAKYLAK--KYNKSE----YIGENILVLDIFFEALNYET 416     |     |
| <i>α_xENaC(model)</i> | 431 GCF TKCRKPCLVSEYQLTAGYSKWPNRVSQDWLHTLSRQYNLTD--RNGIAKLNIFYEELNYKTILESPTINMAMLL 506     |     |
| <i>β_xENaC(model)</i> | 443 NVPCYYSLRDSVAIRENCISLCQQPCNDTHYKMWISMADWPSAGAEDWIFHVLSYEKSSHNITVNRNGIVRLNIYFQE 521     |     |
| <i>γ_xENaC(model)</i> | 440 KVVYKQFVQEELGCQSACRESCSFKEWLTTRSLAKWPSLNSEBWMRLVLSWELGEKLNKLNKTDLANLNIIFYQDLNRS 518    |     |
| <i>α_hENaC(6WTH)</i>  | -----  |     |
| <i>β_hENaC(6WTH)</i>  | 480 FNYRTIEESAA-----   | 490 |
| <i>γ_hENaC(6WTH)</i>  | 463 IMESPA-----  | 468 |
| <i>A_cASIC1(4NTX)</i> | 430 GDIGGQMGFLIGASILTVLELFDYA-----   | 454 |
| <i>B_cASIC1(4NTX)</i> | 412 LNYETIEQKKAYEVAGLLGDIGGQMGFLIGASILTVLELFDYA-----                                       | 454 |
| <i>C_cASIC1(4NTX)</i> | 417 IEQKKAYEVAGLLGDIGGQMGFLIGASILTVLELFDYA-----  | 454 |
| <i>α_xENaC(model)</i> | 507 SLLGSQNSLVFGSSVLSVVEMLELV-----   | 531 |
| <i>β_xENaC(model)</i> | 522 FNYRSISESEATNVWMLLSNLGGQGFWMGGSVLCIEFGEII-----   | 564 |
| <i>γ_xENaC(model)</i> | 519 ISESPTYNIVTLLSNFGGLGLVMSCSMICVLEIEIVFF-----  | 557 |

**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].

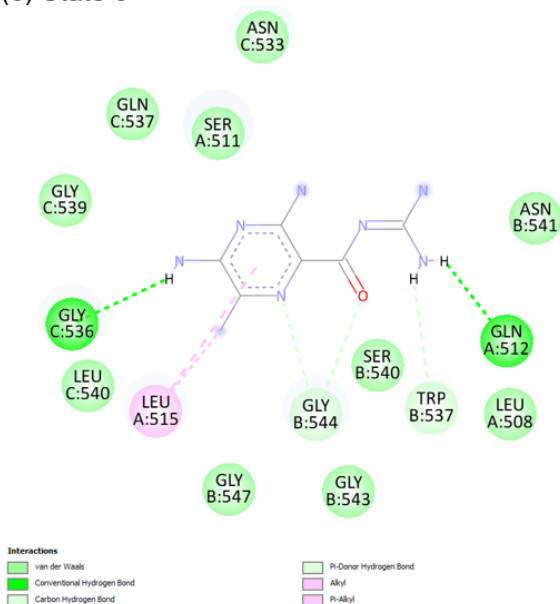
(a) State 1



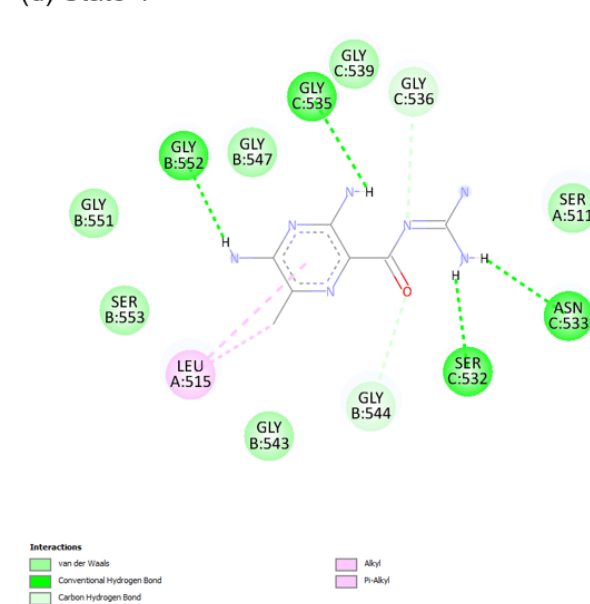
(b) State 2



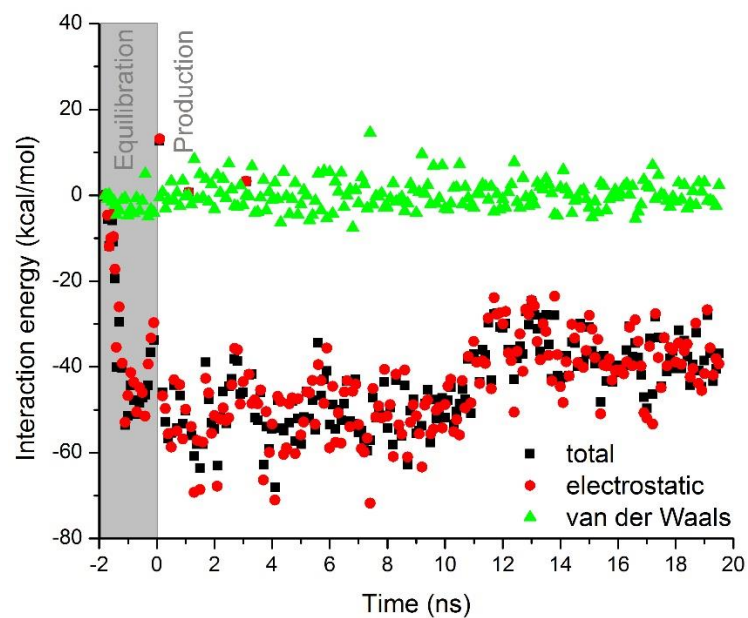
(c) State 3



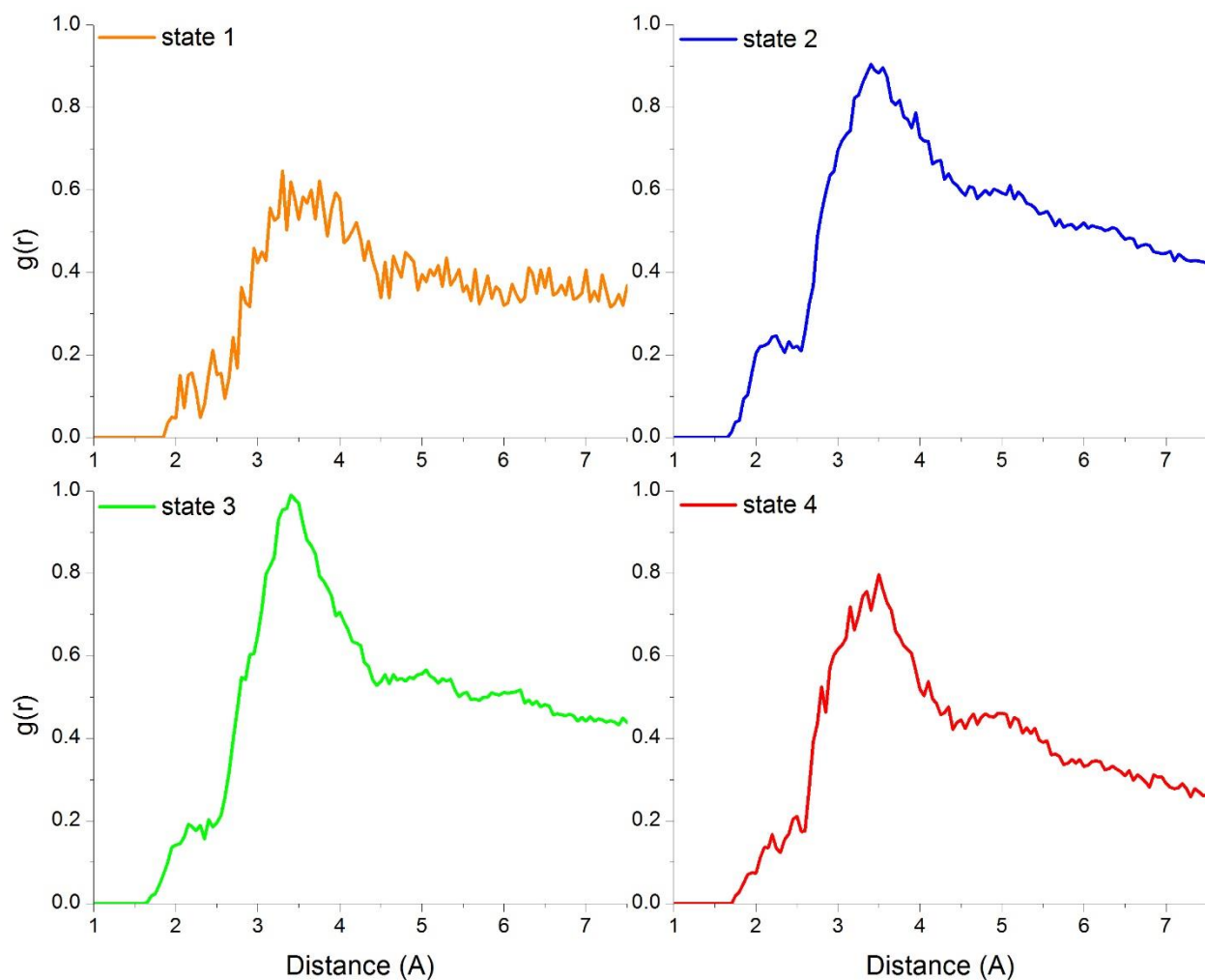
(d) State 4



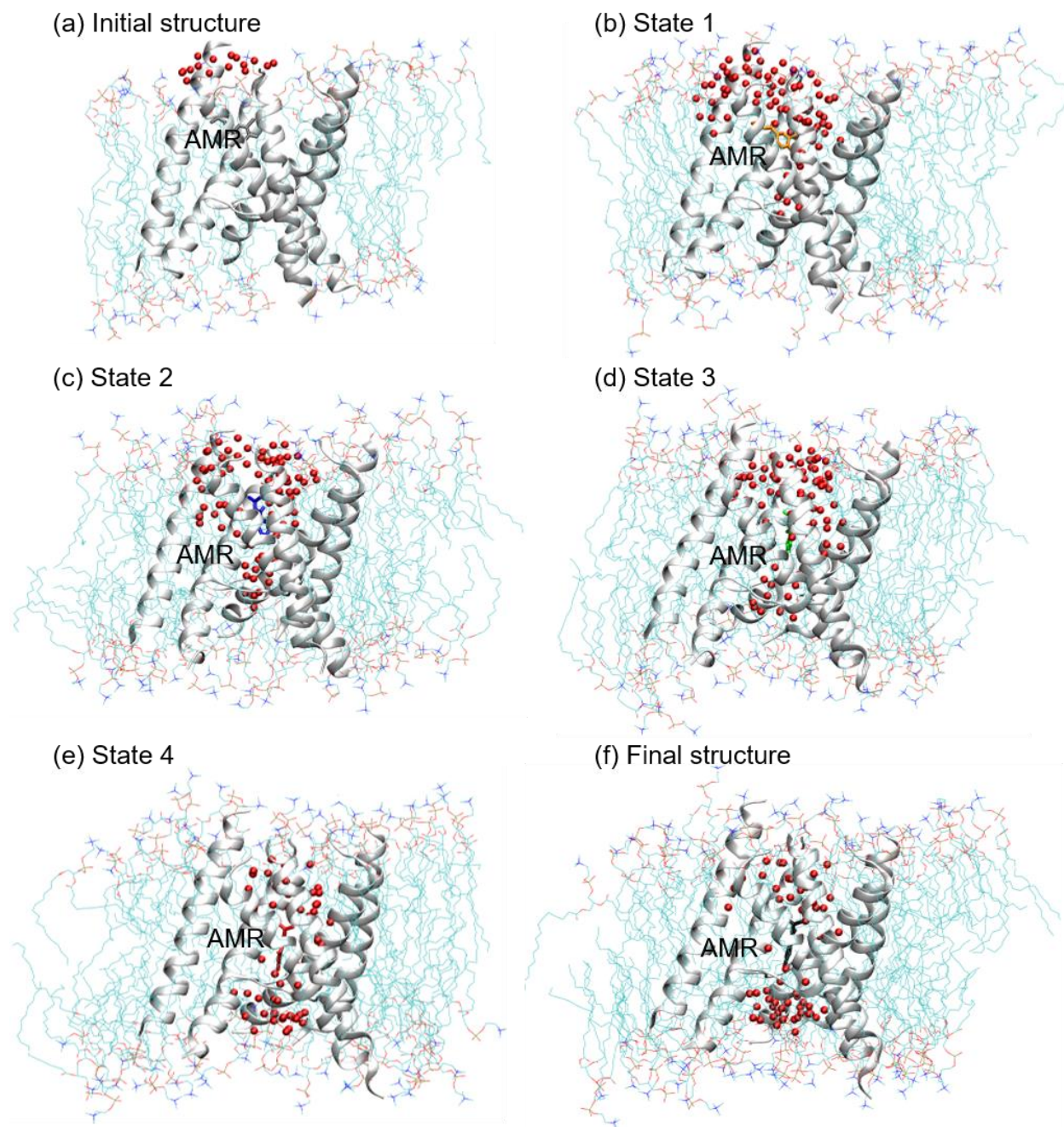
**Figure S2.** 2D interaction maps of amiloride with the representative structures in the four states: (a) state 1 – after ~1.45 ns of equilibration dynamics (-0.5 ns to the production dynamics), (b) state 2 – after ~2 ns of production dynamics, (c) state 3 – after ~10 ns of production dynamics and (d) 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.