SUPPLEMENTAL INFORMATION

Mechanistic insights into the stimulant properties of novel psychoactive substances (NPS) and their discrimination by the dopamine transporter – *in silico* and *in vitro* exploration of dissociative diarylethylamines

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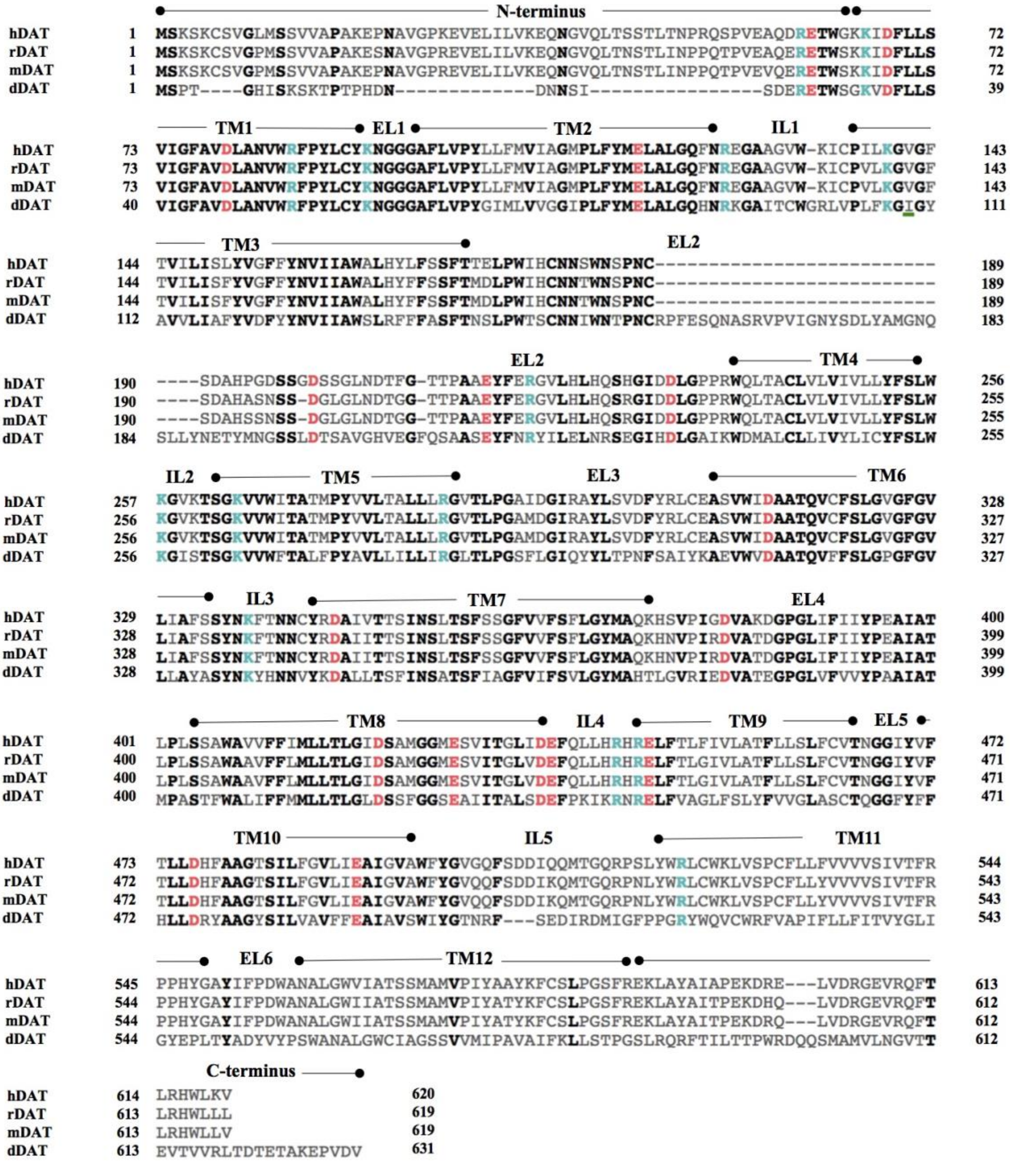
Table S1. The convergence of the calculated free energy change (kcal/mol) in each perturbation for the five compounds in the bound (protein-ligand complex in water) state. (N.B. This is not the change of the binding free energy). The error was estimated based on the standard deviation of all the values used in the estimation of the final value.

Compound	ΔG_4 w.r.t. DPH (kcal/mol) 100ns	ΔG_4 w.r.t. DPH (kcal/mol) 150ns	ΔG_4 w.r.t. DPH (kcal/mol) 175ns	Average ΔG_4 w.r.t. DPH (kcal/mol)
DPH	0	0	0	0
3-MXP	1.19	1.25	1.28	1.25 ± 0.5
4-MXP	1.84	1.89	1.92	1.89 ± 0.5
2-Cl-DPH	-6.77	-6.80	-6.84	-6.84 ± 0.7
2-MXP	15.51	15.59	16.02	15.59 ± 0.8



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                              N-terminus
hDAT    1  MSKSKCSVGLMSSVVAPAKEPNAVGPKEVELILVKEQNGVQLTSSTLTNPRQSPVEAQDRETWGKKIDFLLS   72
rDAT    1  MSKSKCSVGPMSSVVAPAKESNAVGPREVELILVKEQNGVQLTNSTLINPPQTPVEAQERETWSKKIDFLLS   72
mDAT    1  MSKSKCSVGPMSSVVAPAKEPNAVGPREVELILVKEQNGVQLTNSTLINPPQTPVEVQERETWSKKIDFLLS   72
dDAT    1  MSPT----GHISKSKTPTPHDN-------------DNNSI----------------SDERETWSGKVDFLLS   39

              TM1         EL1          TM2                IL1
hDAT   73  VIGFAVDLANVWRFPYLCYKNGGGAFLVPYLLFMVIAGMPLFYMELALGQFNREGAAGVW-KICPILKGVGF  143
rDAT   73  VIGFAVDLANVWRFPYLCYKNGGGAFLVPYLLFMVIAGMPLFYMELALGQFNREGAAGVW-KICPVLKGVGF  143
mDAT   73  VIGFAVDLANVWRFPYLCYKNGGGAFLVPYLLFMVIAGMPLFYMELALGQFNREGAAGVW-KICPVLKGVGF  143
dDAT   40  VIGFAVDLANVWRFPYLCYKNGGGAFLVPYGIMLVVGGIPLFYMELALGQHNRKGAITCWGRLVPLFKGIGY  111

              TM3                                  EL2
hDAT  144  TVILISLYVGFFYNVIIAWALHYLFSSFTTELPWIHCNNSWNSPNC--------------------------  189
rDAT  144  TVILISFYVGFFYNVIIAWALHYFFSSFTMDLPWIHCNNTWNSPNC--------------------------  189
mDAT  144  TVILISFYVGFFYNVIIAWALHYFFSSFTMDLPWIHCNNTWNSPNC--------------------------  189
dDAT  112  AVVLIAFYVDFYYNVIIAWSLRFFFASFTNSLPWTSCNNIWNTPNCRPFESQNASRVPVIGNYSDLYAMGNQ  183

                                   EL2                           TM4
hDAT  190  ----SDAHPGDSSGDSSGLNDTFG-TTPAAEYFERGVLHLHQSHGIDDLGPPRWQLTACLVLVIVLLYFSLW  256
rDAT  190  ----SDAHASNSS-DGLGLNDTGG-TTPAAEYFERGVLHLHQSRGIDDLGPPRWQLTACLVLVIVLLYFSLW  255
mDAT  190  ----SDAHSSNSS-DGLGLNDTGG-TTPAAEYFERGVLHLHQSRGIDDLGPPRWQLTACLVLVIVLLYFSLW  255
dDAT  184  SLLYNETYMNGSSLDTSAVGHVEGFQSAASEYFNRYILELNRSEGIHDLGAIKWDMALCLLIVYLICYFSLW  255

           IL2          TM5                     EL3                      TM6
hDAT  257  KGVKTSGKVVWITATMPYVVLTALLLRGVTLPGAIDGIRAYLSVDFYRLCEASVWIDAATQVCFSLGVGFGV  328
rDAT  256  KGVKTSGKVVWITATMPYVVLTALLLRGVTLPGAMDGIRAYLSVDFYRLCEASVWIDAATQVCFSLGVGFGV  327
mDAT  256  KGVKTSGKVVWITATMPYVVLTALLLRGVTLPGAMDGIRAYLSVDFYRLCEASVWIDAATQVCFSLGVGFGV  327
dDAT  256  KGISTSGKVVWFTALFPYAVLLILLIRGLTLPGSFLGIQYYLTPNFSAIYKAEVWVDAATQVFFSLGPGFGV  327

                 IL3                TM7                     EL4
hDAT  329  LIAFSSYNKFTNNCYRDAIVTTSINSLTSFSSGFVVFSFLGYMAQKHSVPIGDVAKDGPGLIFIIYPEAIAT  400
rDAT  328  LIAFSSYNKFTNNCYRDAIITTSINSLTSFSSGFVVFSFLGYMAQKHNVPIRDVATDGPGLIFIIYPEAIAT  399
mDAT  328  LIAFSSYNKFTNNCYRDAIITTSINSLTSFSSGFVVFSFLGYMAQKHNVPIRDVATDGPGLIFIIYPEAIAT  399
dDAT  328  LLAYASYNKYHNNVYKDALLTSFINSATSFIAGFVIFSVLGYMAHTLGVRIEDVATEGPGLVFVVYPAAIAT  399

                       TM8               IL4       TM9          EL5
hDAT  401  LPLSSAWAVVFFIMLLTLGIDSAMGGMESVITGLIDEFQLLHRHRELFTLFIVLATFLLSLFCVTNGGIYVF  472
rDAT  400  LPLSSAWAAVFFLMLLTLGIDSAMGGMESVITGLVDEFQLLHRHRELFTLGIVLATFLLSLFCVTNGGIYVF  471
mDAT  400  LPLSSAWAAVFFLMLLTLGIDSAMGGMESVITGLVDEFQLLHRHRELFTLGIVLATFLLSLFCVTNGGIYVF  471
dDAT  400  MPASTFWALIFFMMLLTLGLDSSFGGSEAIITALSDEFPKIKRNRELFVAGLFSLYFVVGLASCTQGGFYFF  471

              TM10                  IL5                        TM11
hDAT  473  TLLDHFAAGTSILFGVLIEAIGVAWFYGVGQFSDDIQQMTGQRPSLYWRLCWKLVSPCFLLFVVVVSIVTFR  544
rDAT  472  TLLDHFAAGTSILFGVLIEAIGVAWFYGVQQFSDDIKQMTGQRPNLYWRLCWKLVSPCFLLYVVVVSIVTFR  543
mDAT  472  TLLDHFAAGTSILFGVLIEAIGVAWFYGVQQFSDDIKQMTGQRPNLYWRLCWKLVSPCFLLYVVVVSIVTFR  543
dDAT  472  HLLDRYAAGYSILVAVFFEAIAVSWIYGTNRF---SEDIRDMIGFPPGRYWQVCWRFVAPIFLLFITVYGLI  543

               EL6          TM12
hDAT  545  PPHYGAYIFPDWANALGWVIATSSMAMVPIYAAYKFCSLPGSFREKLAYAIAPEKDRE---LVDRGEVRQFT  613
rDAT  544  PPHYGAYIFPDWANALGWIIATSSMAMVPIYATYKFCSLPGSFREKLAYAITPEKDHQ---LVDRGEVRQFT  612
mDAT  544  PPHYGAYIFPDWANALGWIIATSSMAMVPIYATYKFCSLPGSFREKLAYAITPEKDRQ---LVDRGEVRQFT  612
dDAT  544  GYEPLTYADYVYPSWANALGWCIAGSSVVMIPAVAIFKLLSTPGSLRQRFTILTTPWRDQQSMAMVLNGVTT  612

               C-terminus
hDAT  614  LRHWLKV                  620
rDAT  613  LRHWLLL                  619
mDAT  613  LRHWLLV                  619
dDAT  613  EVTVVRLTDTETAKEPVDV      631
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Figure S1: Alignment of human (hDAT), rat (rDAT), mouse (mDAT) and fruit-fly (dDAT) amino acid sequences. Conservation > 99% is indicated in black and highlights the high conservation at the transmembrane (TM) regions, especially TM1-10. Positively charged residues are coloured in red and negatively charged residues in cyan. The figure was prepared using Alignment-Annotator web server [42].

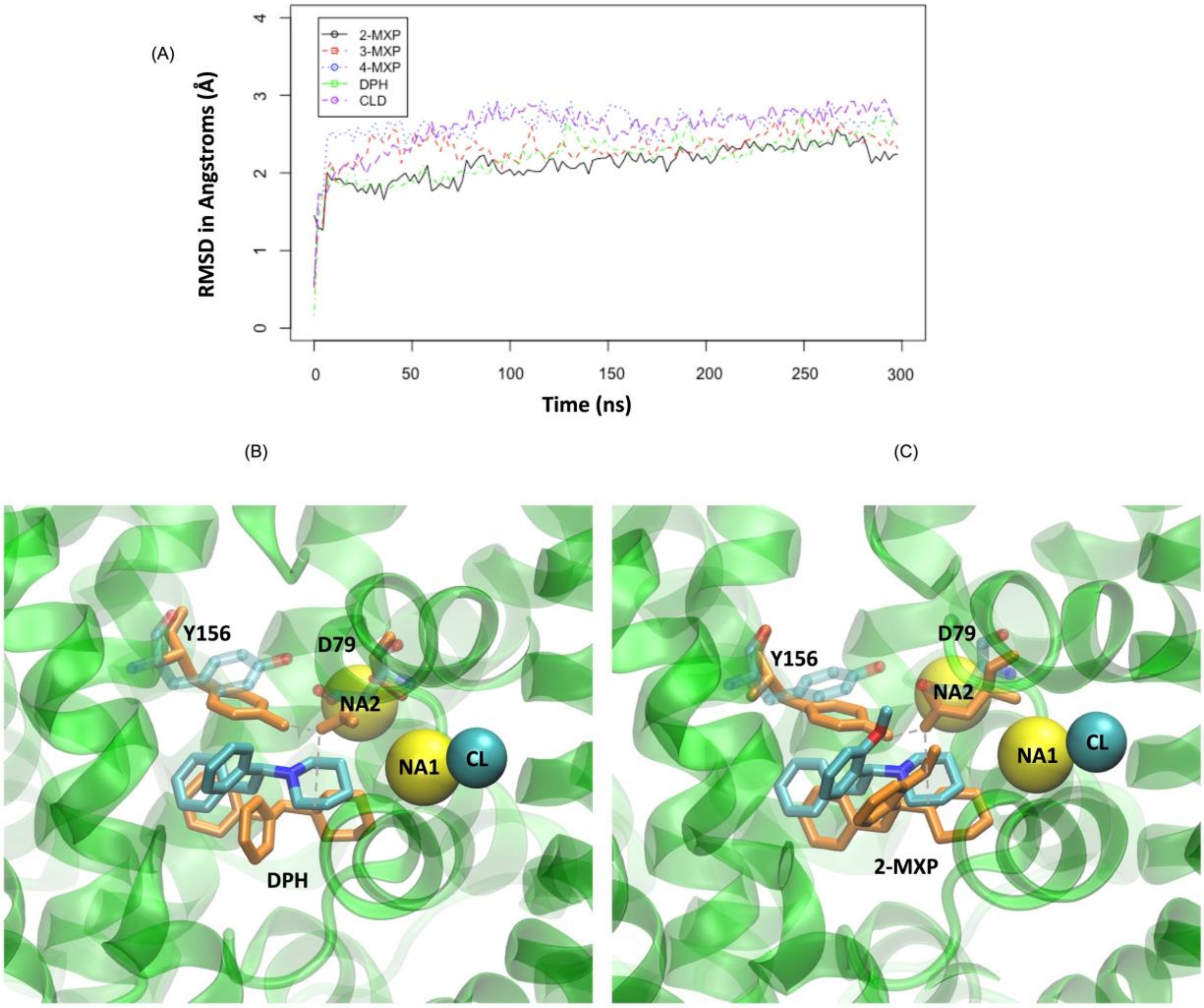


Figure S2: (A) RMSD graph and superimposition of compounds at the binding site after docking (cyan) and after 100ns (orange) of unbiased MD for (B) DPH and (C) 2-MXP. Internal sodium and chloride ions are depicted as yellow and cyan coloured spheres. Dashed lines refer to the salt bridge interaction between Asp79 and the protonated amine of the respective compound as well as the hydrogen bond between the OH group of Tyr156 and Asp79.

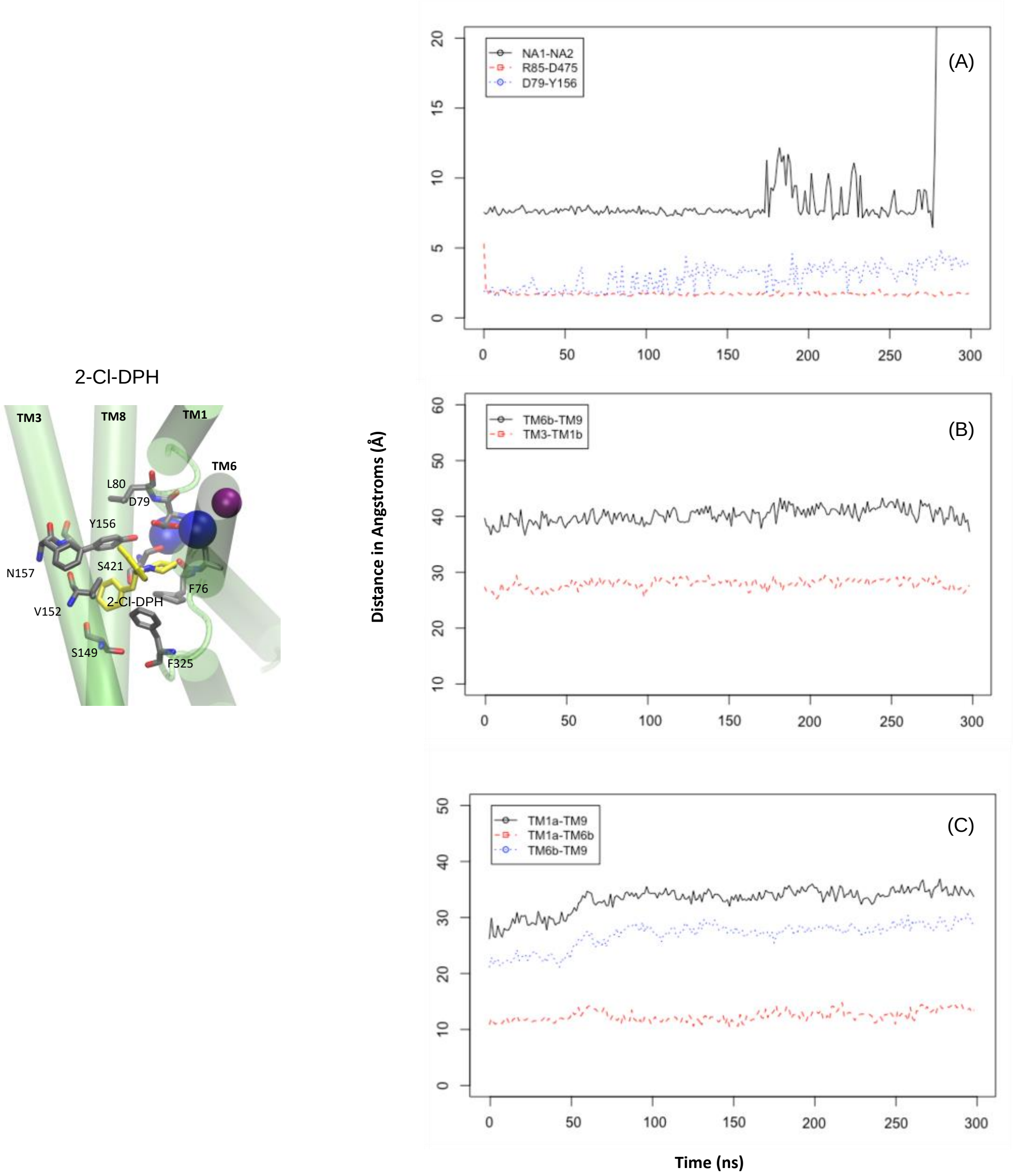
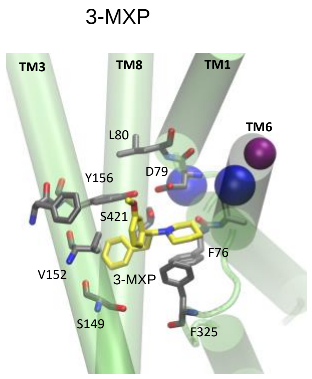


Figure S3: Time evolution in the simulations for rDAT when bound to 2-Cl-DPH. (A) depicts the distances between D79 and Y156, Na1 and Na2 and R85 and D475. (B) and (C) depict the Cβ-Cβ distances between residues in various extracellular and intracellular transmembrane (TM) segments respectively for I67 (in TM1a) and L446 (in TM9), I67 (in TM1a) and S332 (in TM6b) and S332 (in TM6b) and L446 (in TM9); E306 (in TM6a) and F171 (in TM3); and F171 (in TM3) and K92 (in TM1b).



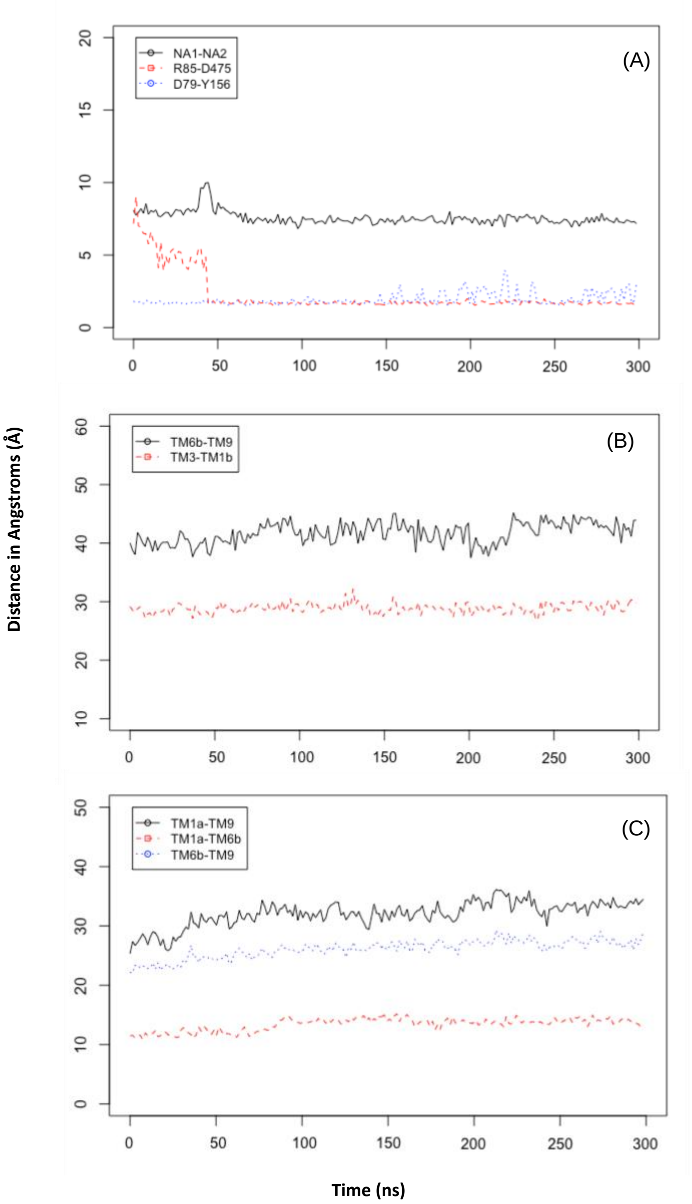
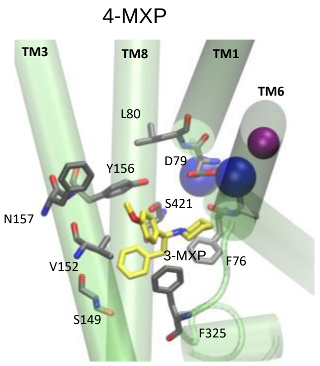


Figure S4: Time evolution in the simulations for rDAT when bound to 3-MXP. (A) depicts the distances between D79 and Y156, Na1 and Na2 and R85 and D475. (B) and (C) depict the Cβ-Cβ distances between residues in various extracellular and intracellular transmembrane (TM) segments respectively for I67 (in TM1a) and L446 (in TM9), I67 (in TM1a) and S332 (in TM6b) and S332 (in TM6b) and L446 (in TM9); E306 (in TM6a) and F171 (in TM3); and F171 (in TM3) and K92 (in TM1b).



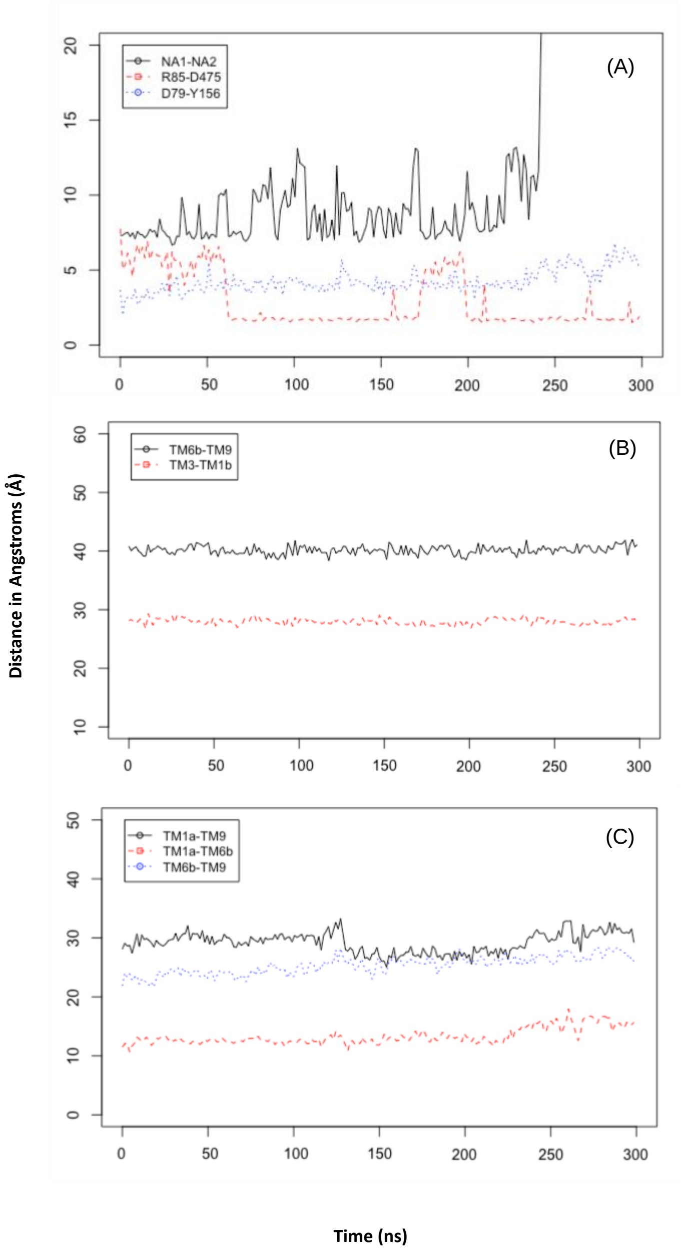


Figure S5: Time evolution in the simulations for rDAT when bound to 4-MXP. (A) depicts the distances between D79 and Y156, Na1 and Na2 and R85 and D475. (B) and (C) depict the Cβ-Cβ distances between residues in various extracellular and intracellular transmembrane (TM) segments respectively for I67 (in TM1a) and L446 (in TM9), I67 (in TM1a) and S332 (in TM6b) and S332 (in TM6b) and L446 (in TM9); E306 (in TM6a) and F171 (in TM3); and F171 (in TM3) and K92 (in TM1b).