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Supplemental Information

A Dynamic Picture of the Early Events in Nociceptin Binding to the NOP Receptor by Metadynamics

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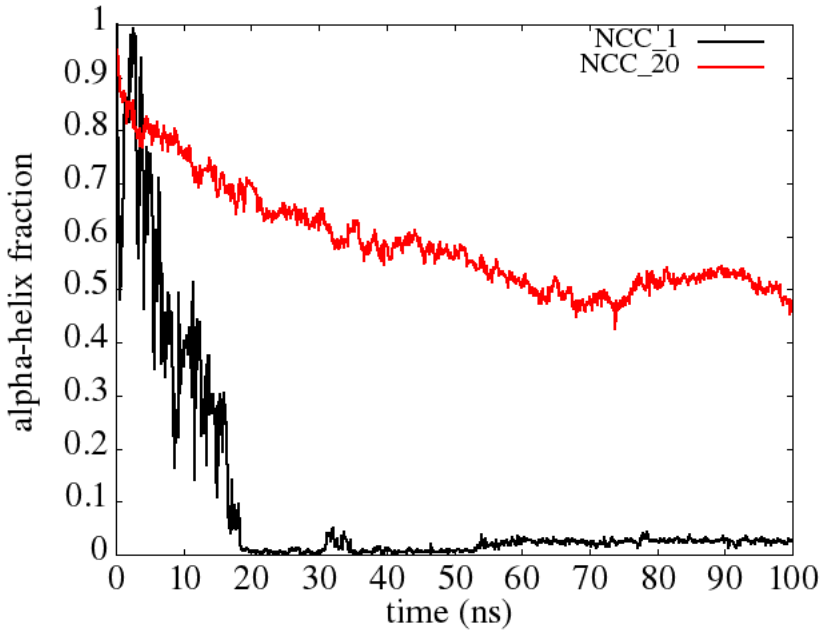


Fig. S1. Fraction of α-helix content of NCC in water starting either from a single molecule (black curve), or a cluster of 20 NCC molecules (red curve) per 1600 water molecules. A partial α-helix content is mantained much longer for clustered NCC molecules

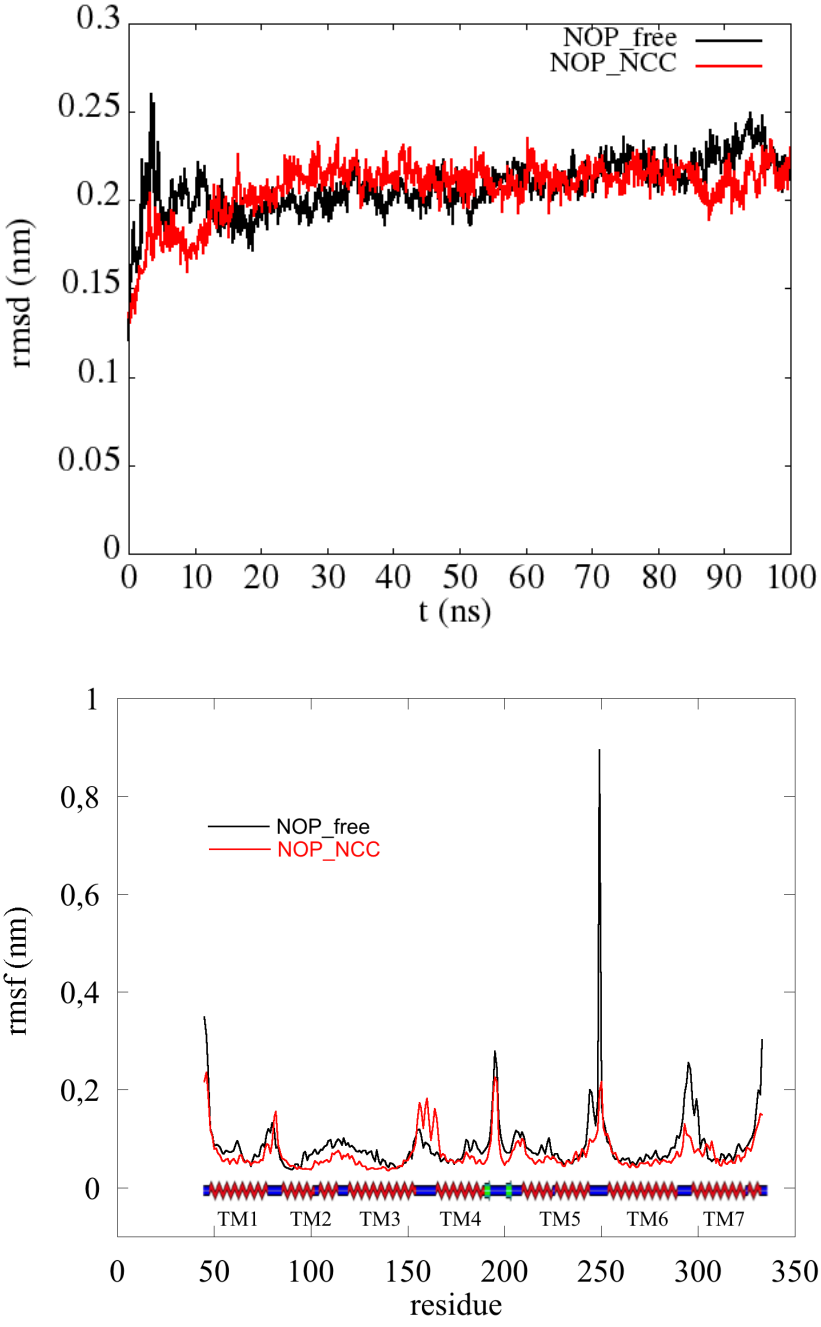
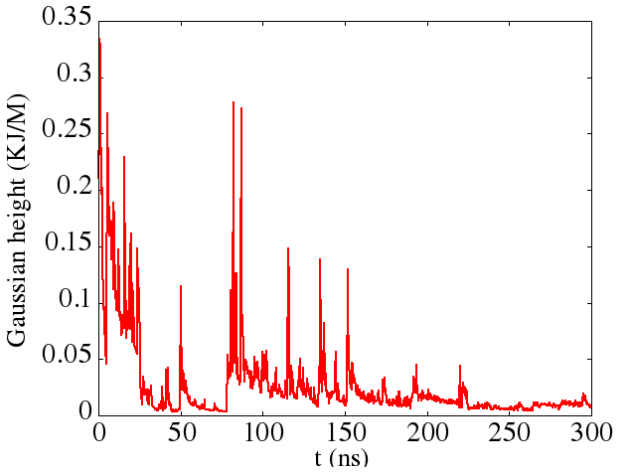
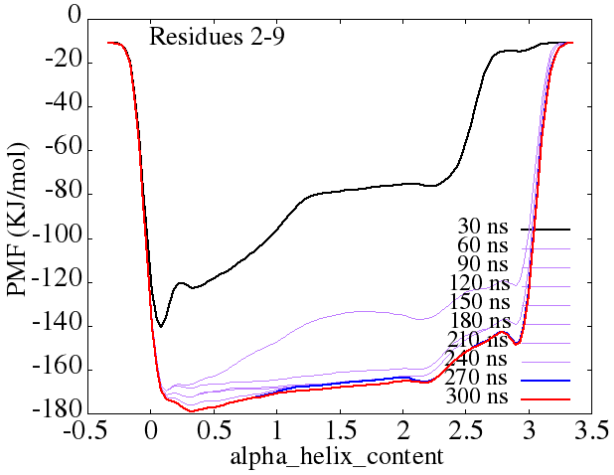


Fig. S2. Upper panel) RMSD plot of a 100 ns trajectory for free (black curve) and NCC bound (red curve) NOP. Lower panel) RMSF of residues of the same molecules. The NOP_NCC structure is the starting one obtained by rigid docking, before metadynamics.

A)



B)



C)

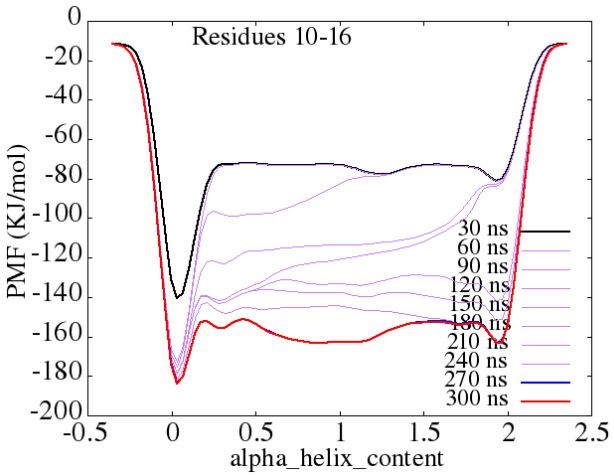


Fig. S3. Well-tempered metadynamics of the NOP_NCC complex as a function of NCC α-helix content. A) Gaussian heights added along the trajectory. B) and C) Projections of the FES averaged along the first and second collective variable, respectively.

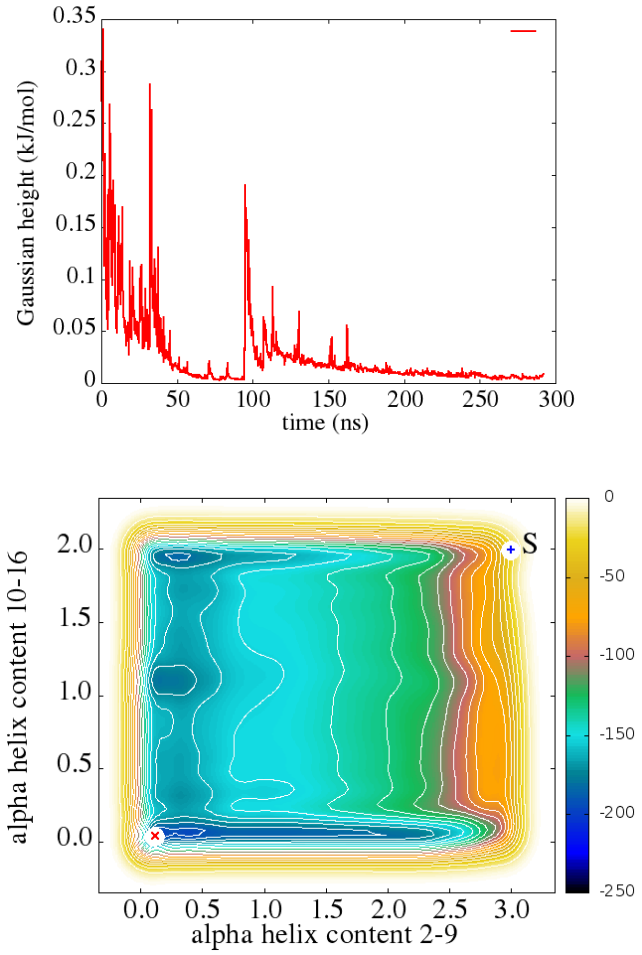


Fig. S4. Well-tempered metadynamics of the N133A 'in silico' mutant as a function of NCC α-helix content. A) Gaussian heights added along the trajectory. B) Bidimensional FES .

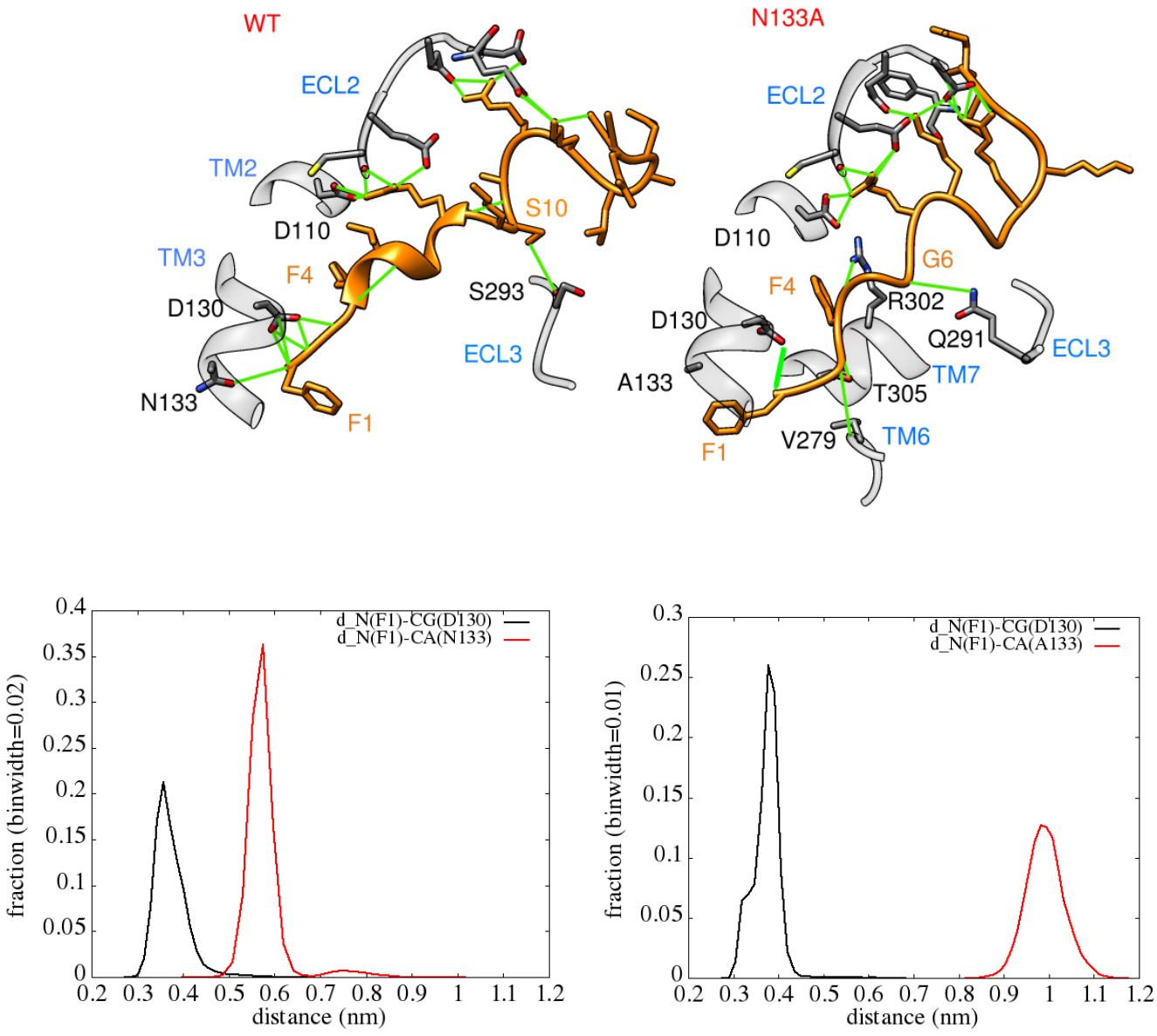


Fig. S5. Comparison between the representative structures corresponding to the FES absolute minima (State_1) obtained for the WT (left) and N133A 'in silico' mutant (right) NOP-NCC complex. The distributions of the distances dN(F1)-CG(D130) and dN(F1)-CA(N/A133) along the metadynamics trajectory is shown in the bottom frames, showing that in the absence of the N133 bond, NCC still forms H-bond with D130, but penetrates less inside the binding pocket.

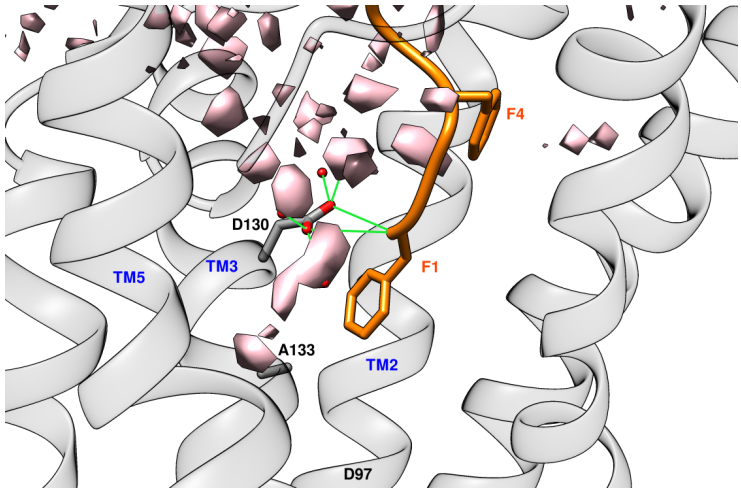


Fig. S6. Water density (pink) and H-bond network calculated for the N133A NOP-NCC complex: In the absence of the N(F1)-OD1(N133) bond, water penetrates deeper inside the binding site, restoring interactions with D130.

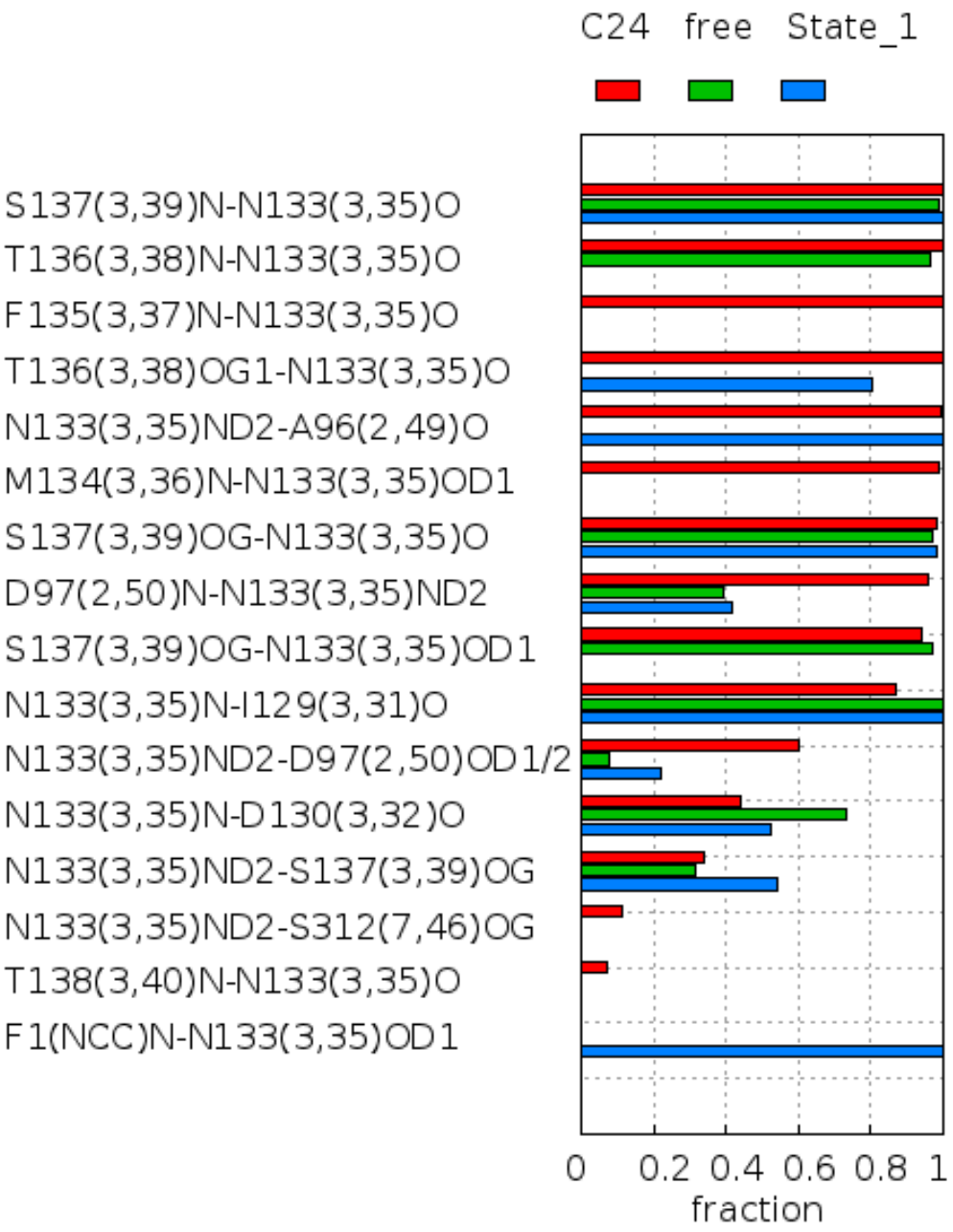


Fig. S7. Hydrogen bonds formed by N133 along the trajectories of NOP-C24 complex (red), NOP_free (green) and NOP-NCC (cyan). Values of persistence of the H-bonds in the histogram are expressed as fraction of the trajectory. The histogram enlightens the destabilization of H-bonds between N133 and (M134, F135, T136) i.e. the perturbation of the TM3 helix segment in the NOP-NCC complex

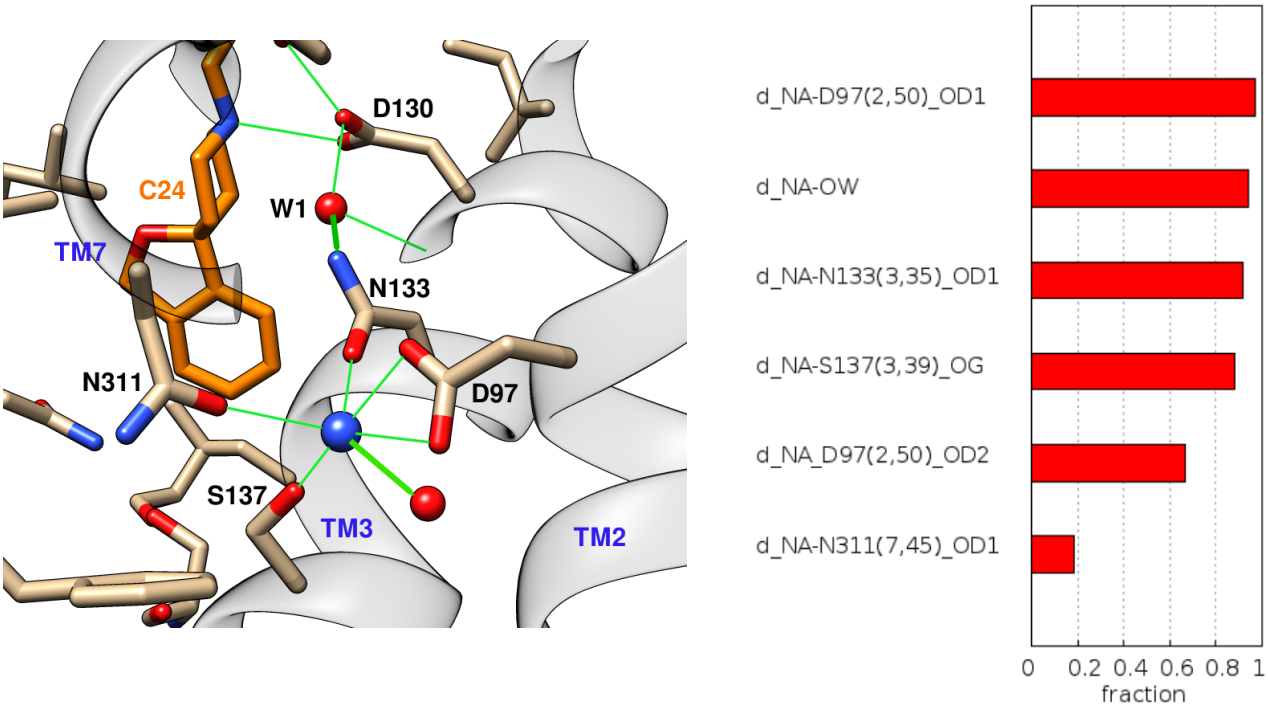


Fig. S8. Stability of the proposed allosteric binding site of sodium (colored blue), as probed by 100 ns MD of the NOP_C24 complex: the sodium ion has been put in place of water W2 (left panel). As shown in the right panel, it forms a quite stable (91%) salt bridge with N133(3,35), with D97(2,50) (OD1 97%, OD2 67%), S137(3,39)_OG (88%), N311(7,45)_OD1 (18%), and with another structural water molecule (93%).