

Fig. S7-1. Simulations of MOP- μ OR and HMP- μ OR: the W293 side chain orientation. (A) Basically, the W293 dihedral angle $chi^{2,1}$ fluctuates around two favorable values ($\sim 0^\circ$ in MOP- μ OR and $\sim 120^\circ$ in HMP- μ OR). The $chi^{2,1}$ value is plotted as a function of simulation time in (B, C) the two independent MD simulations for each system and in (D) the forward and backward alchemical free energy perturbations in the bound state.

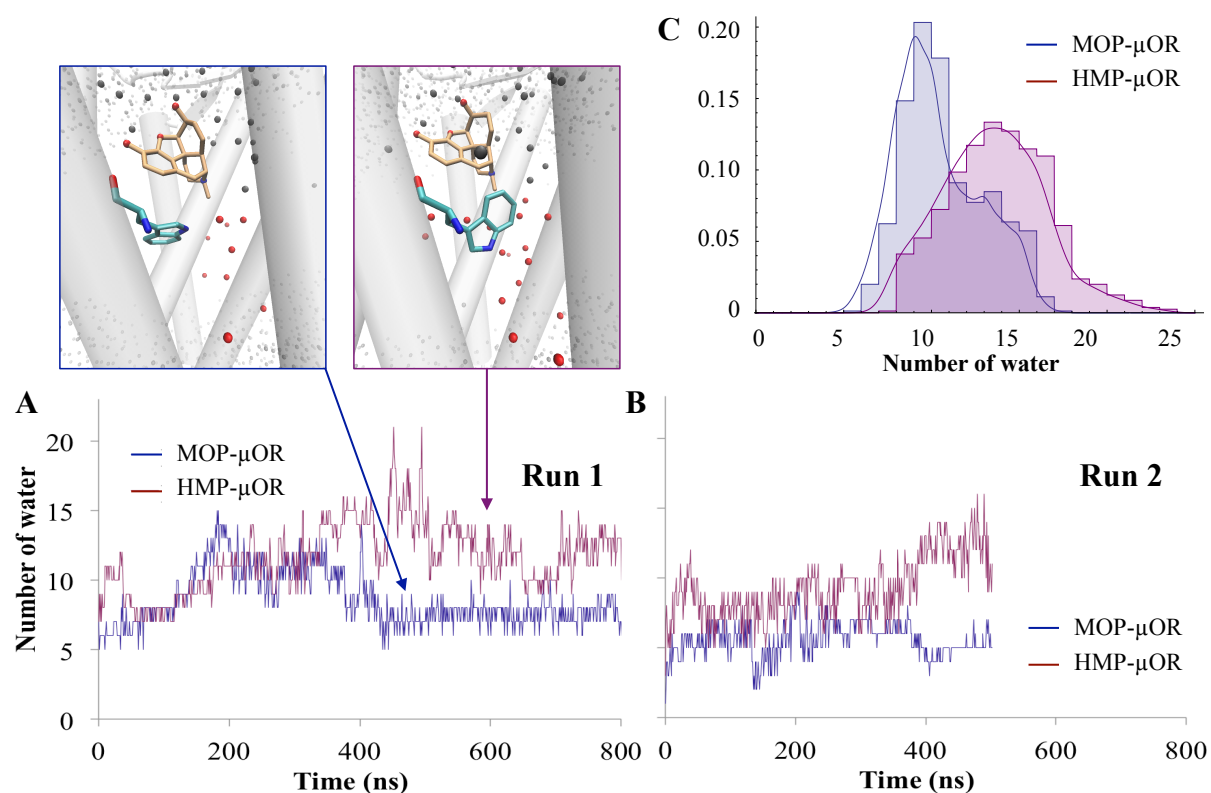


Fig. S7-2. MD simulations of MOP- μ OR and HMP- μ OR: number of water molecules inside the μ OR binding cavity. (A, B) The number plotted as a function of time in the two independent MD simulations. (C) Normalized histograms of the data plotted in (A).

We performed Welch's t -test on the two sets of data plotted in Fig. S7-2A. The results (Table S7-1) indicate that the difference shown in Fig. S7-2A is statistically significant.

Table S7-1. Welch's t -test^a on the data shown in Fig. S7-2A.

P-value ^b	Difference in mean values	Standard error	95% confidence interval of the difference	Intermediate values used in the test	
				t	df
< 0.0001	3.086	0.133	2.825–3.347	23.2261	1541

^aThe test assumes normal distribution of the two datasets. Although this is not the case for MOP, the test has been shown to be still robust with a large sample size [1] (i.e. ≥ 200 [2]).

^bP-value much less than the threshold (0.05) indicates significant difference between the two datasets.

Table S7-2. Distances (\AA) between the agonists and selected protein residues averaged over two independent MD runs.

	MD run	I296	M151	G325	I322
MOP	Run 1 (0.8 μ s)	2.4 \pm 0.2	2.2 \pm 0.2	2.4 \pm 0.3	3.3 \pm 0.5
	Run 2 (0.5 μ s)	2.4 \pm 0.2	2.2 \pm 0.2	2.9 \pm 0.7	2.3 \pm 0.2
HMP	Run 1 (0.8 μ s)	2.4 \pm 0.2	2.3 \pm 0.2	5.0 \pm 0.3	2.7 \pm 0.2
	Run 2 (0.5 μ s)	2.6 \pm 0.3	2.1 \pm 0.2	5.8 \pm 0.4	2.6 \pm 0.3

Supporting references

1. Fagerland MW. t-tests, non-parametric tests, and large studies--a paradox of statistical practice? *BMC medical research methodology*. 2012;12:78. Epub 2012/06/16. doi: 10.1186/1471-2288-12-78. PubMed PMID: 22697476; PubMed Central PMCID: PMC3445820.
2. Fagerland MW, Sandvik L. Performance of five two-sample location tests for skewed distributions with unequal variances. *Contemp Clin Trials*. 2009;30(5):490-6. doi: Doi 10.1016/J.Cct.2009.06.007. PubMed PMID: ISI:000270073800018.