

SI Appendix

Dissecting the innate immune recognition of morphine and its metabolites by TLR4/MD2: an *in silico* simulation study

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Table S1. MM/PBSA-derived binding free energies (kcal/mol) for protonated opioids binding to (TLR4/MD2)₂

	ΔE_{vdW}	ΔE_{cle}	$\Delta G_{\text{sol-polar}}$	$\Delta G_{\text{sol-nonpolar}}$	$\Delta G_{\text{binding}}$
protonated morphine	-33.9±0.3	-48.6±0.5	51.8±0.4	21.3±0.2	-9.4±0.3
protonated M3G	-45.7±0.4	-47.8±0.4	53.3±0.3	28.9±0.2	-11.2±0.4
protonated M6G	-41.2±0.7	-65.9±0.5	64.1±0.4	25.0±0.4	-17.9±0.5

Numbers after ± present standard errors of mean

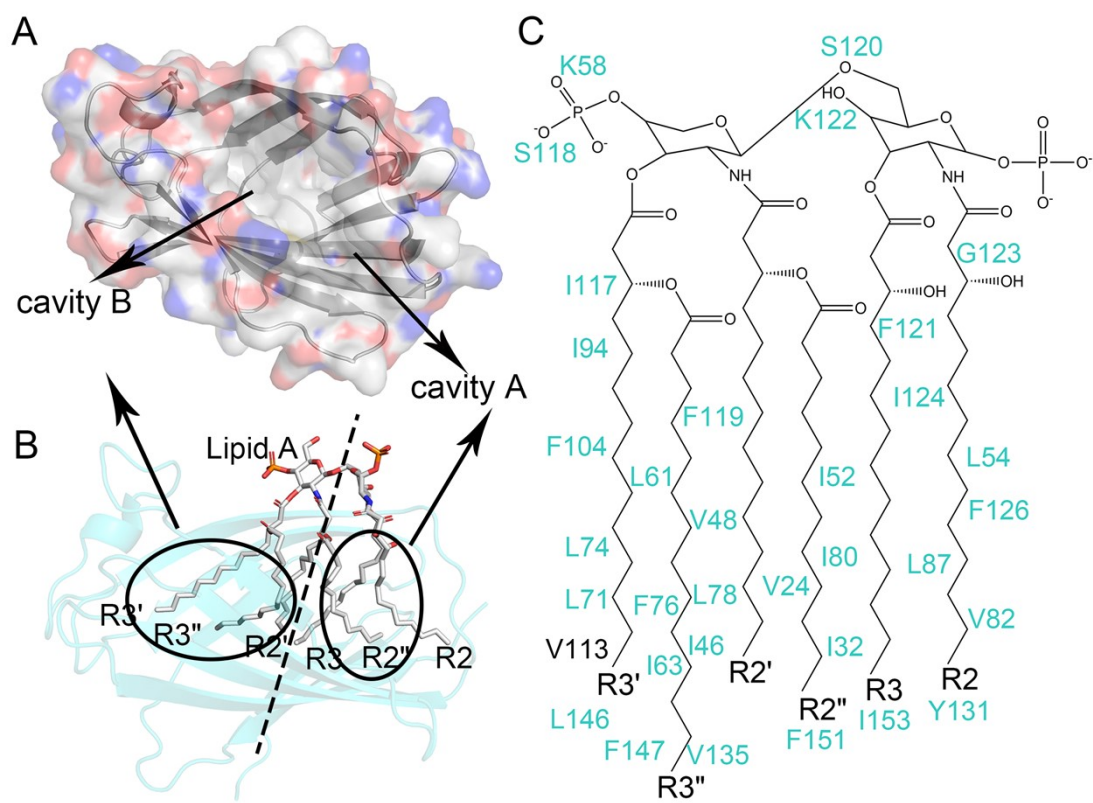


Figure S1. (A) The binding pocket of MD2 can be divided into cavity A and cavity B. (B) The lipid chains of lipid A interact with the hydrophobic pocket in MD2. (C) Chemical structure of the lipid A. MD2 residues involved in the interactions with the lipid chains were labeled.

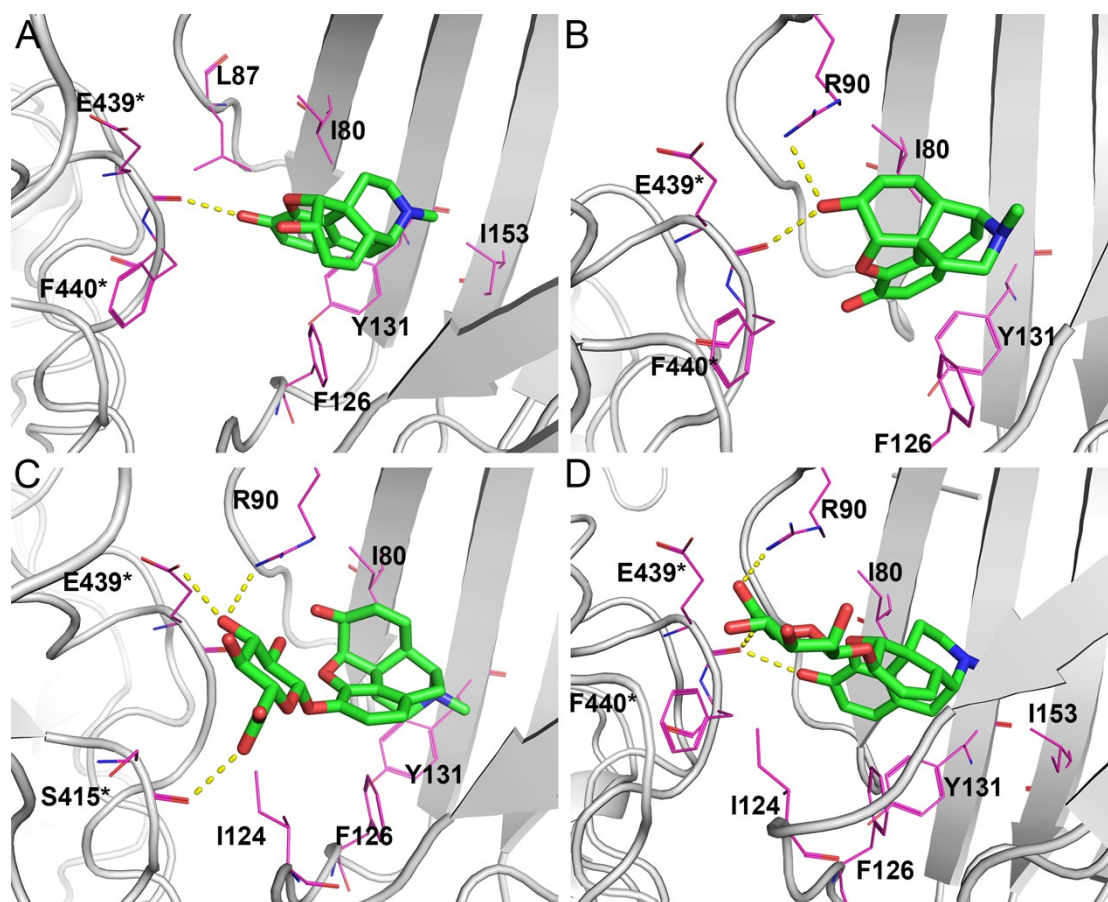


Figure S2. The key interactions corresponding to morphine (A), (+)-morphine (B), M3G (C), and M6G (D) docking with (TLR4/MD2)₂.

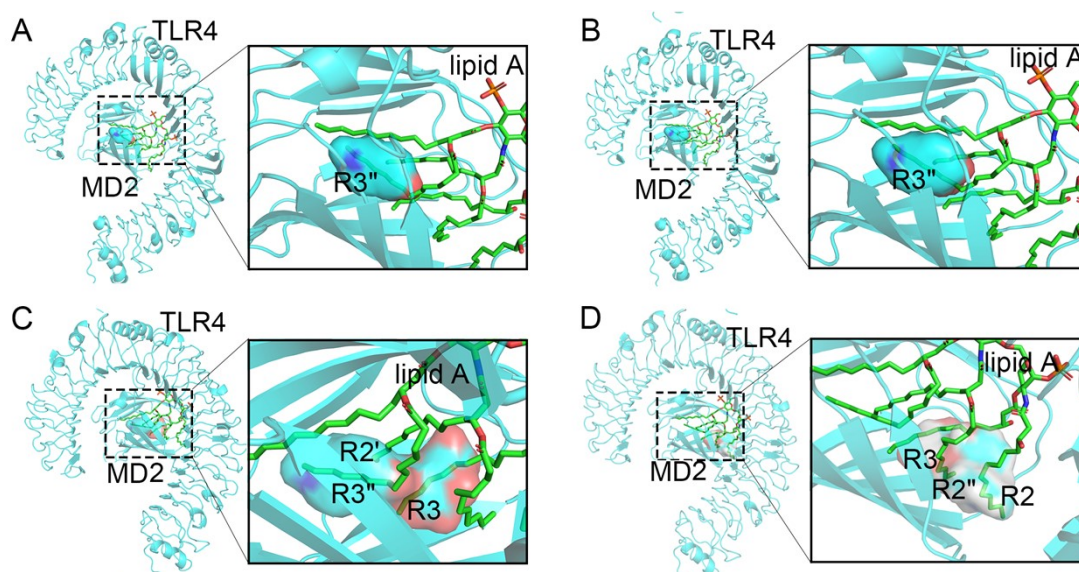


Figure S3. Overlap of the best docking pose of morphine and its metabolites with lipid A in the heterodimeric (TLR4/MD2)₂ systems. Morphine (A) and (+)-morphine (B) overlapped with the R3'' chain of lipid A; M3G (C) overlapped with the R2', R3, and R3'' chains of lipid A and occupied a large portion of MD2; M6G (D) overlapped with the R2, R2'', and R3 chains of lipid A. MD2 was shown as a cyan cartoon; lipid A was shown as a green stick model; ligands were shown as a magenta surface models.

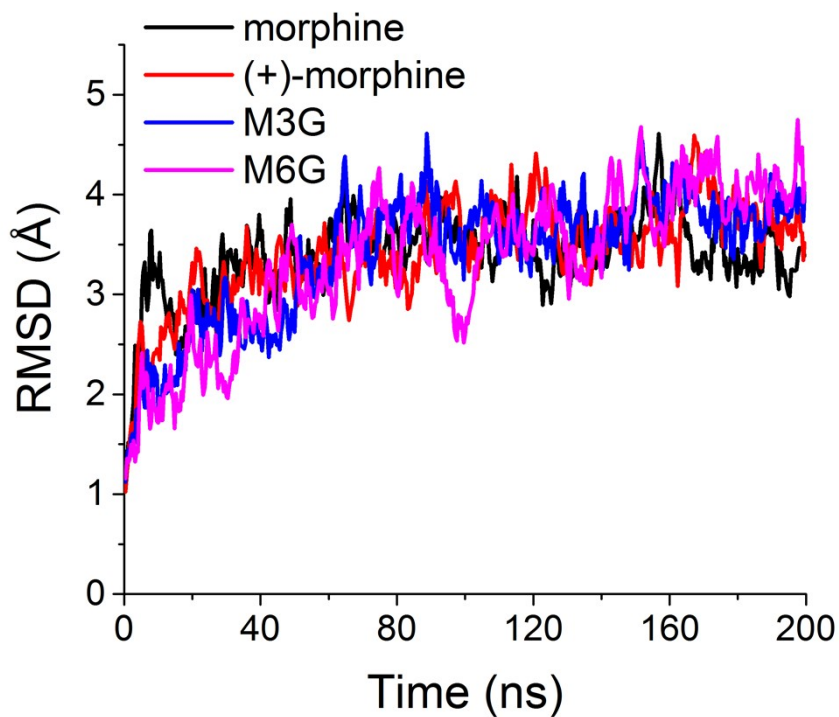


Figure S4. Time evolution of the (TLR4/MD2)₂ backbone RMSDs during molecular dynamics simulations. Black, red, blue, and magenta indicated (TLR4/MD2)₂ bound with morphine, (+)-morphine, M3G, and M6G, respectively.

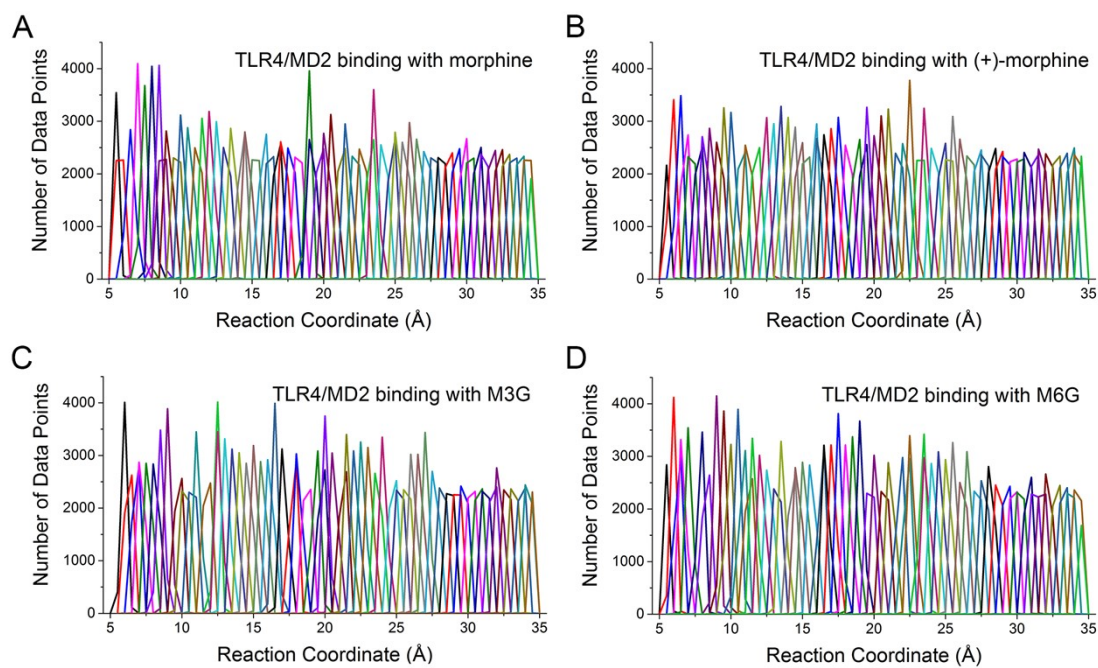


Figure S5. Histogram analysis of the overlap between umbrella windows along the reaction coordinate for (TLR4/MD2)₂ binding with morphine (A), (+)-morphine (B), M3G (C), and M6G. Each window consistently overlapped with its neighboring windows.

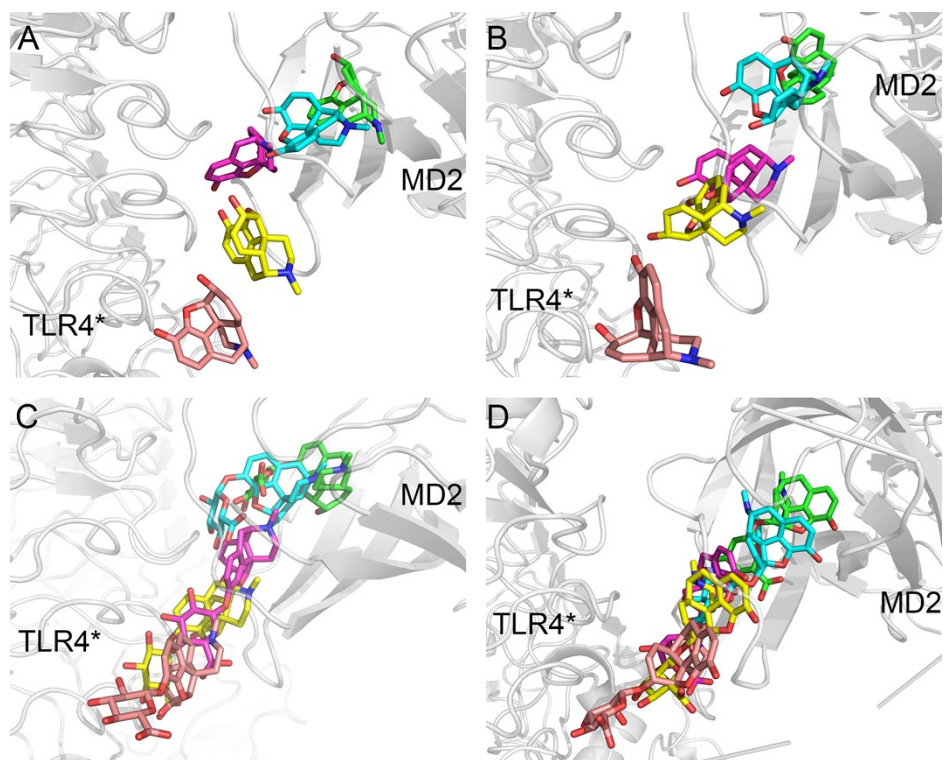


Figure. S6. Binding pathways of morphine (A), (+)-morphine (B), M3G (C), and M6G (salmon → yellow →magenta → cyan → green) with (TLR4/MD2)₂.

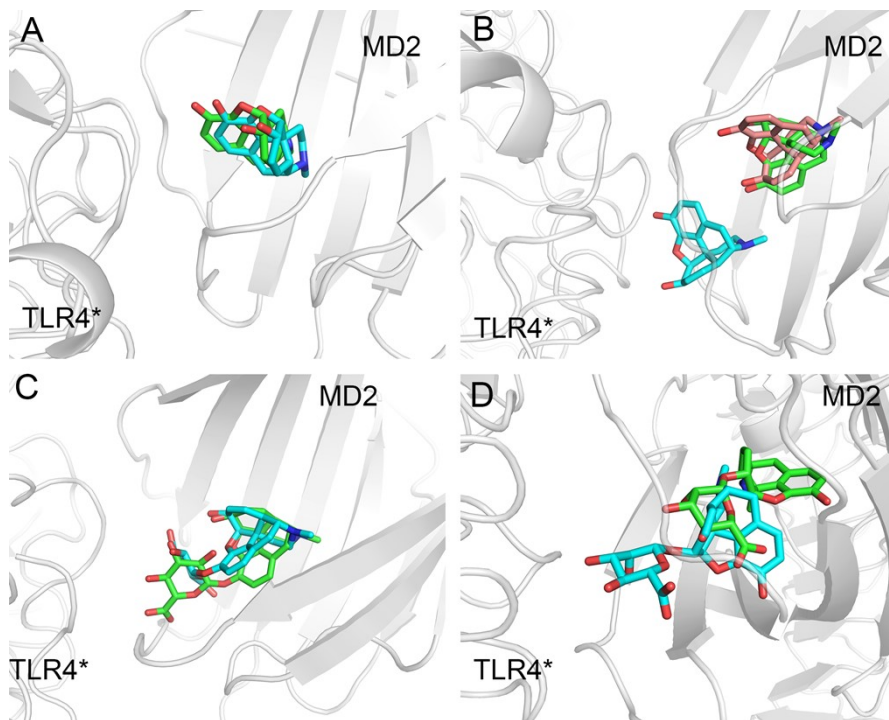


Figure S7. Alignment of conformations of morphine (A), (+)-morphine (B), M3G (C), and M6G (D) in the lowest free energy frame of molecular dynamics simulations (green), and the minima of PMFs (cyan). The intermediate conformation of (+)-morphine was shown as salmon stick.

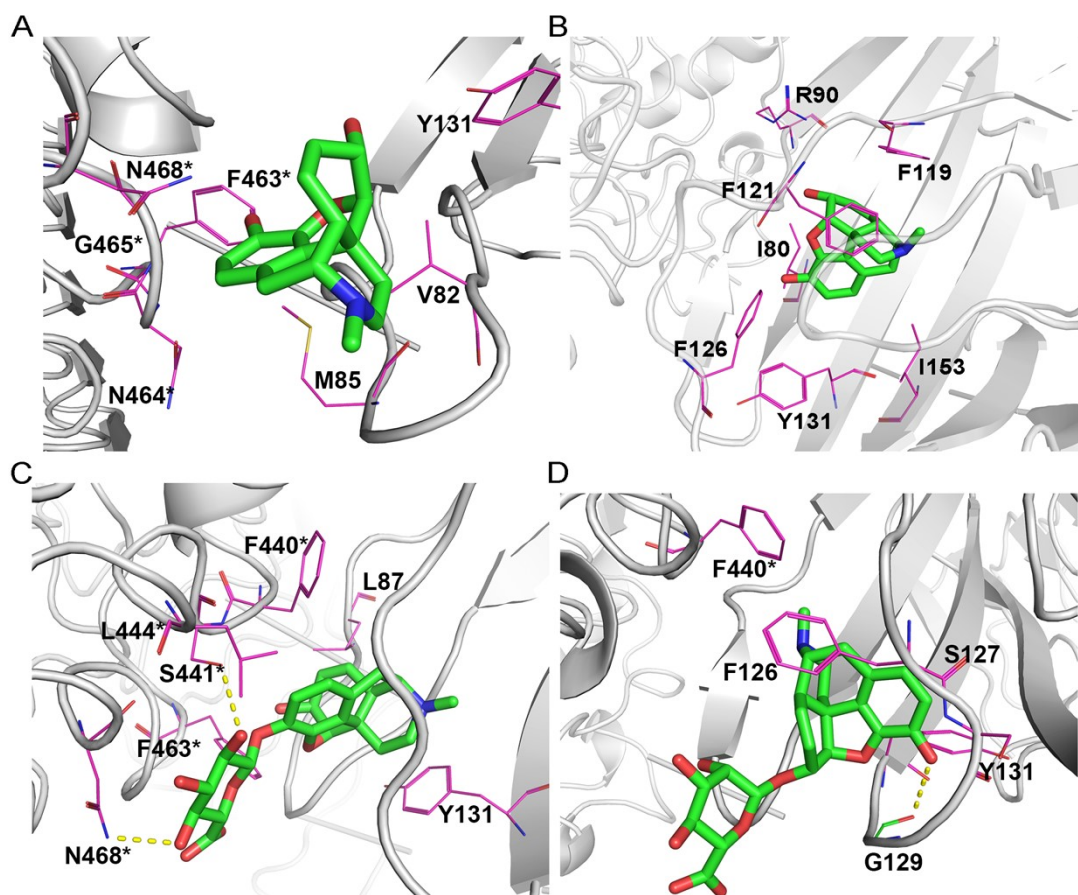


Figure S8. The intermediate states corresponding to morphine (A), (+)-morphine (B), M3G (C), and M6G (D) binding with $(TLR4/MD2)_2$. Ligands were shown as green stick models. TLR4* and MD2 were shown as grey cartoons. The key residues in interacting with ligands during the binding process were shown as magenta sticks.