Supporting Information

A comprehensive evaluation of the potential binding poses of fentanyl and its analogs at the μ-opioid receptor

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Supporting Tables

Table S1. The relative free energies of the minima identified on the free energy surfaces (FESs) rebuilt from the metadynamics simulation results. The energy of the global minimum on a FES is defined as 0. The relative energetic differences of other minima and their corresponding CV1 and CV2 values are listed. The results of calculations using both OPLS4 (left) and OPLS3e (right) are shown.

| Ligand             | OPLS4                  | OPLS3                  |
|--------------------|------------------------|------------------------|
|                    | state | CV1  | CV2  | minimum (kcal/mol) | state | CV1  | CV2  | minimum (kcal/mol) |
| fentanyl           | TL    | 165  | -80  | 0.0              | TL    | -160 | -50  | 0.0              |
|                    | TH    | -175 | 110  | 0.8              | TH    | -170 | 105  | 0.7              |
|                    | CL    | 0    | -85  | 2.3              | CL    | -5   | -85  | 2.2              |
|                    | CH    | 0    | 110  | 4.2              | CH    | 5    | 110  | 4.5              |
| carfentanil        | TL2   | 165  | -105 | 0.0              | TL2   | 170  | -110 | 0.0              |
|                    | TL1   | -170 | -10  | 0.3              | TL1   | -170 | -10  | 0.2              |
|                    | CL2   | 0    | -110 | 5.9              | CL2   | -5   | -110 | 5.7              |
|                    | CL1   | 5    | -10  | 5.6              | CL1   | 5    | -10  | 5.8              |
| (3S, 4R)-lofentanil| TL2   | 175  | -120 | 0.0              | TL2   | 175  | -120 | 0.0              |
|                    | TL1   | -170 | -5   | 6.8              | TL1   | -170 | -5   | 6.9              |
|                    | CL2   | 0    | -120 | 5.8              | CL2   | 0    | -120 | 5.7              |
|                    | CL1   | 0    | 0    | 13.3             | CL1   | 0    | 0    | 13.4             |
| (3R, 4S)-lofentanil| TL2   | 170  | -115 | 5.8              | TL2   | 175  | -120 | 5.8              |
|                    | TL1   | -180 | 0    | 0.0              | TL1   | -180 | 0    | 0.0              |
|                    | CL2   | -5   | -115 | 12.2             | CL2   | 0    | -120 | 12.4             |
|                    | CL1   | 0    | 0    | 6.0              | CL1   | 0    | 0    | 5.9              |
| (3S, 4R)-3-methyl- | TL    | -165 | -120 | 0.0              | TL    | -165 | -120 | 0.1              |
| fentanyl           | TH    | -160 | 75   | 0.1              | TH    | -160 | 75   | 0.0              |
|                    | CL    | 5    | -100 | 3.2              | CL    | 0    | -90  | 3.3              |
|                    | CH    | 5    | 80   | 5.3              | CH    | 5    | 80   | 5.1              |
| (3R, 4S)-3-methyl- | TL    | 165  | -15  | 0.1              | TL    | 165  | -15  | 0.1              |
| fentanyl           | TH    | 160  | 145  | 0.0              | TH    | 160  | 145  | 0.0              |
|                    | CL    | 0    | -45  | 3.2              | CL    | 0    | -40  | 3.1              |
|                    | CH    | -5   | 135  | 5.3              | CH    | -5   | 135  | 5.3              |
Table S2. The energy barriers (EB) of the selected minimum free energy paths (MFEPs) on the FESs of fentanyl and its analogs. See methods for the protocol to identify the MFEP between two minima (states) on a FES. EB is defined as the difference between the starting state and the vertex with the highest energy on the MFEP. While the MFEP between two states is unique, the value of EB depends on the starting state, i.e., either A=>B or B=>A.

| Ligand          | state A | state B | highest on MFEP | EB (A=>B) | EB (B=>A) |
|-----------------|---------|---------|-----------------|-----------|-----------|
| **OPLS4**       |         |         |                 |           |           |
| fentanyl        | TL      | TH      | 6.5             | 6.5       | 5.7       |
|                 | TL      | CL      | 14.8            | 14.8      | 12.5      |
|                 | CL      | CH      | 13.0            | 10.7      | 8.8       |
|                 | TH      | CH      | 14.8            | 14.0      | 10.6      |
| carfentanil     | TL2     | TL1     | 8.9             | 8.9       | 8.6       |
|                 | CL2     | CL1     | 16.1            | 16.1      | 10.2      |
|                 | TL1     | CL1     | 16.3            | 16.0      | 10.7      |
| (3S, 4R)-lofental | TL2   | CL2     | 17.1            | 17.1      | 11.3      |
|                 | CL2     | CL1     | 20.4            | 14.6      | 7.1       |
|                 | TL1     | CL1     | 20.4            | 13.6      | 7.1       |
| (3R, 4S)-lofental | TL2 | TL1     | 16.5            | 10.7      | 16.5      |
|                 | CL2     | CL1     | 19.8            | 14.0      | 7.6       |
|                 | TL1     | CL1     | 19.8            | 7.6       | 13.8      |
| (3S, 4R)-3-methyl-fentanyl | TL | TH      | 6.9             | 6.9       | 6.8       |
|                 | TL      | CL      | 14.0            | 14.0      | 10.8      |
|                 | CL      | CH      | 13.7            | 10.5      | 8.4       |
|                 | TH      | CH      | 14.0            | 13.9      | 8.7       |
| (3R, 4S)-3-methyl-fentanyl | TL | TH      | 6.8             | 6.7       | 6.8       |
|                 | TL      | CL      | 13.9            | 13.9      | 10.7      |
|                 | CL      | CH      | 13.5            | 10.3      | 8.2       |
|                 | TH      | CH      | 13.9            | 13.9      | 8.6       |
| **OPLS3**       |         |         |                 |           |           |
| fentanyl        | TL      | TH      | 6.5             | 6.5       | 5.8       |
|                 | TL      | CL      | 14.8            | 14.8      | 12.6      |
|                 | CL      | CH      | 12.9            | 10.7      | 8.4       |
|                 | TH      | CH      | 14.8            | 14.1      | 10.3      |
| carfentanil     | TL2     | TL1     | 8.5             | 8.5       | 8.3       |
|                 | CL2     | CL1     | 16.0            | 16.0      | 10.3      |
|                 | TL1     | CL1     | 16.2            | 16.0      | 10.4      |
| (3S, 4R)-lofental | TL2   | CL2     | 15.8            | 15.8      | 8.9       |
|                 | CL2     | CL1     | 20.5            | 14.8      | 7.1       |
|                 | TL1     | CL1     | 20.5            | 13.6      | 7.1       |
| (3R, 4S)-lofental | TL2 | TL1     | 16.4            | 10.6      | 16.4      |
|                 | CL2     | CL1     | 19.8            | 14.0      | 7.4       |
|                 | CL2     | CL1     | 19.8            | 7.4       | 13.9      |
| (3S, 4R)-3-methyl-fentanyl | TL | TH      | 6.8             | 6.7       | 6.8       |
|                 | TL      | CL      | 14.0            | 13.9      | 10.7      |
|                 | CL      | CH      | 13.4            | 10.1      | 8.3       |
|                 | TH      | CH      | 14.0            | 14.0      | 8.9       |
| (3R, 4S)-3-methyl-fentanyl | TL | TH      | 6.6             | 6.5       | 6.6       |
|                 | TL      | CL      | 14.0            | 13.9      | 10.9      |
|                 | CL      | CH      | 13.6            | 10.5      | 8.3       |
|                 | TH      | CH      | 14.0            | 14.0      | 8.7       |
Table S3. Molecular dynamics (MD) simulations summary. APF and FPA represent two opposite orientations of fentanyl binding pose in the MOR binding site (Fig. 1B,C). The “T” and “C” in the subscript stand for trans and cis conformations of fentanyl, respectively (Fig. 1D). The “E” or “D” in the subscript denotes that the His$^{6.52}$ is in either the HIE or HID protonation form, respectively.

| Condition               | Force Field   | APF$_{ET}$ | APF$_{DT}$ | APF$_{EC}$ | APF$_{DC}$ |
|-------------------------|---------------|------------|------------|------------|------------|
|                         | num trajs     | length (μs)| num trajs  | length (μs)| num trajs  | length (μs)| num trajs  | length (μs)| num trajs  | length (μs)| num trajs  | length (μs)|
| Fentanyl                | OPLS3e        | 16         | 22.5       | 15         | 19.3       | 7          | 8.9        | 3          | 6.0        |
|                         | OPLS4         | 3          | 3.6        | 3          | 9.0        | 5          | 7.8        | 3          | 3.6        |
| Carfentanil             | OPLS3e        | 3          | 3.6        | 3          | 3.6        | 3          | 3.6        | 3          | 5.8        |
|                         | OPLS4         | 3          | 3.6        | 3          | 3.6        |            |            |            |            |
| (3S,4R)-Lofentanil      | OPLS3e        | 3          | 3.6        | 3          | 3.6        | 3          | 3.6        | 3          | 5.2        |
|                         | OPLS4         | 3          | 3.5        | 3          | 3.6        |            |            |            |            |
| (3R,4S)-Lofentanil      | OPLS3e        | 3          | 3.6        | 3          | 3.6        |            |            |            |            |
|                         | OPLS4         | 3          | 3.6        | 3          | 3.6        |            |            |            |            |
| DAMGO                   | OPLS3e        | 17         | 20.3       |            |            |            |            |            |            |
|                         | OPLS4         | 6          | 7.2        |            |            |            |            |            |            |
Table S4. The mutagenesis results related to the binding of fentanyl and its analogs at the MOR in the literature. We extracted the data from the GPCRdb and our own literature review and filtered the Ki binding data for single mutation without considering truncation. The Ki fold difference between the mutant (mut) and wild type (wt) larger than 4, or smaller than 0.5, are highlighted in red and green, respectively.

| mutation position | BW number | mut from | mut to | ligand name | exp type | probe | wt value (nM) | mut value (nM) | fold | reference |
|-------------------|-----------|----------|--------|-------------|----------|-------|---------------|---------------|------|-----------|
| 148               | 3.33      | Y        | F      | ohmefentanyl (RTI4614-4) | Ki       | [125I]IOXY | 0.59           | 2.71           | 4.59 | (Xu et al., 1999) |
| 148               | 3.33      | Y        | F      | ohmefentanyl (2S,3R,4S)-1a | Ki       | [125I]IOXY | 0.41           | 0.73           | 1.78 | (Xu et al., 1999) |
| 148               | 3.33      | Y        | F      | ohmefentanyl (2R,3R,4S)-1b | Ki       | [125I]IOXY | 0.78           | 5.25           | 6.73 | (Xu et al., 1999) |
| 148               | 3.33      | Y        | F      | ohmefentanyl (2R,3S,4R)-1c | Ki       | [125I]IOXY | 205.00         | 980.00         | 4.78 | (Xu et al., 1999) |
| 148               | 3.33      | Y        | F      | ohmefentanyl (2S,3S,4R)-1d | Ki       | [125I]IOXY | 96.60          | 574.00         | 5.94 | (Xu et al., 1999) |
| 150               | 3.35      | N        | A      | fentanyl   | Ki       | [3H]-bremazocine | 41.63           | 2.04           | 0.05 | (Mansour et al., 1997) |
| 198               | 4.56      | I        | V      | fentanyl   | Ki       | [3H]-bremazocine | 41.63           | 61.01         | 1.47 | (Mansour et al., 1997) |
| 202               | 4.60      | V        | I      | fentanyl   | Ki       | [3H]-bremazocine | 41.63           | 40.34         | 0.97 | (Mansour et al., 1997) |
| 297               | 6.52      | H        | N      | sufentanyl | Ki       | [3H]naloxone  | 1.30           | 0.80           | 0.62 | (Spivak et al., 1997) |
| 297               | 6.52      | H        | Q      | sufentanyl | Ki       | [3H]naloxone  | 1.30           | 1.10           | 0.85 | (Spivak et al., 1997) |
| 303               | 6.58      | K        | E      | fentanyl   | Ki       | [3H]-bremazocine | 14.50           | 16.30         | 1.12 | (Bonner et al., 2000) |
| 303               | 6.58      | K        | Q      | fentanyl   | Ki       | [3H]-bremazocine | 14.50           | 27.70         | 1.91 | (Bonner et al., 2000) |
| 303               | 6.58      | K        | W      | fentanyl   | Ki       | [3H]-bremazocine | 14.50           | 42.30         | 2.92 | (Bonner et al., 2000) |
| 318               | 7.35      | W        | K      | fentanyl   | Ki       | [3H]-bremazocine | 14.50           | 7.00           | 0.48 | (Bonner et al., 2000) |
| 318               | 7.35      | W        | L      | fentanyl   | Ki       | [3H]-bremazocine | 14.50           | 26.50         | 1.83 | (Bonner et al., 2000) |
| 319               | 7.36      | H        | A      | ohmefentanyl (RTI4614-4) | Ki       | [125I]IOXY | 0.59           | 20.50         | 34.75 | (Xu et al., 1999) |
| 319               | 7.36      | H        | A      | ohmefentanyl (2S,3R,4S)-1a | Ki       | [125I]IOXY | 0.41           | 6.83           | 16.66 | (Xu et al., 1999) |
| 319               | 7.36      | H        | A      | ohmefentanyl (2R,3R,4S)-1b | Ki       | [125I]IOXY | 0.78           | 21.30         | 27.31 | (Xu et al., 1999) |
| 319               | 7.36      | H        | A      | ohmefentanyl (2R,3S,4R)-1c | Ki       | [125I]IOXY | 205.00         | 1404.00       | 6.85  | (Xu et al., 1999) |
| 319               | 7.36      | H        | A      | ohmefentanyl (2S,3S,4R)-1d | Ki       | [125I]IOXY | 96.60          | 960.00        | 9.94  | (Xu et al., 1999) |
| 326               | 7.43      | Y        | F      | fentanyl   | Ki       | [3H]-bremazocine | 41.63           | 3000.00       | 72.06 | (Mansour et al., 1997) |

*all the residue numbers are those of rat MOR.*
Supporting Figures and Figure Legends

Figure S1. The chemical structures of fentanyl and its analogs investigated in this study.
Figure S2. Metadynamics simulations of fentanyl and its analogs using the OPLS4 force field. See Fig. 2 for the legend.
Figure S3. The standard deviations of the FESs reconstructed from the metadynamics simulations results. The top and bottom rows are the results from the simulations using the OPLS3e and OPLS4 force fields, respectively, for the indicated ligands.
Figure S4. The interaction between the amide carbonyl and Gln126$^{2.60}$ or Asn129$^{2.63}$ contributes to stabilizing the FPA$_{DC}$ condition. For each of the indicated fentanyl analogs, evolutions of the CV1 dihedral angle (upper row) and the minimum distance between the amide carbonyl and the sidechain of Gln126$^{2.60}$ or Asn129$^{2.63}$ of hMOR (lower row) are shown along the MD simulation trajectories using the OPLS3e force field.
Figure S5. The HIE form of His299$^{6.52}$ is more dynamic than the HID form in the MD simulation of the fentanyl bound hMOR-Gi complexes. The distribution of the $\chi_1$ rotamer of His299$^{6.52}$ is plotted against that of the $\chi_2$ rotamer of Trp295$^{6.48}$. The results of the simulations using the OPLS4 force field are shown for each indicated condition. The APF$_{ET}$ and FPA$_{EC}$ conditions have more sparse distributions than those of APF$_{DT}$ and FPA$_{DC}$. In addition, the two His299$^{6.52}$ protonation forms also result in slightly different $\chi_2$ dihedral angles of Trp295$^{6.48}$. 

![diagram showing the distribution of rotamers](image-url)
Figure S6. The distributions of the CV1 and CV2 dihedral angles of fentanyl analogs in the MD simulation trajectories using the OPLS3e force field. Only the relatively stable conditions APF_{ET}, APF_{DT}, FPA_{EC}, and FPA_{DC} are shown.
Figure S7. The distributions of the CV1 and CV2 dihedral angles of fentanyl analogs in the MD simulation trajectories using the OPLS4 force field. Only the relatively stable conditions APF$_{ET}$, APF$_{DT}$, FPA$_{EC}$, and FPA$_{DC}$ are shown.
Figure S8. The DAMGO-bound hMOR-Gi complex is highly stable in our MD simulations and its conformation is similar to that revealed by the cryo-EM structure. The evolutions of the RMSDs based on the Cα atoms of the transmembrane region were calculated against the cryo-EM structure of the mouse MOR (PDB 6DDF) in OPLS4 RMSDs. The results are shown for three replicas of the simulations with the His299\(^{6,52}\) in its HIE (A) or HID (B) protonation state using the OPLS4 force field.
References

Bonner, G., Meng, F., and Akil, H. (2000). Selectivity of mu-opioid receptor determined by interfacial residues near third extracellular loop. Eur J Pharmacol 403, 37-44. 10.1016/s0014-2999(00)00578-1.

Mansour, A., Taylor, L.P., Fine, J.L., Thompson, R.C., Hoversten, M.T., Mosberg, H.I., Watson, S.J., and Akil, H. (1997). Key residues defining the mu-opioid receptor binding pocket: a site-directed mutagenesis study. J Neurochem 68, 344-353. 10.1046/j.1471-4159.1997.68010344.x.

Spivak, C.E., Beglan, C.L., Seidleck, B.K., Hirshbein, L.D., Blaschak, C.J., Uhl, G.R., and Surratt, C.K. (1997). Naloxone activation of mu-opioid receptors mutated at a histidine residue lining the opioid binding cavity. Mol Pharmacol 52, 983-992. 10.1124/mol.52.6.983.

Xu, H., Lu, Y.F., Partilla, J.S., Zheng, Q.X., Wang, J.B., Brine, G.A., Carroll, F.I., Rice, K.C., Chen, K.X., Chi, Z.Q., and Rothman, R.B. (1999). Opioid peptide receptor studies, 11: involvement of Tyr148, Trp318 and His319 of the rat mu-opioid receptor in binding of mu-selective ligands. Synapse 32, 23-28. 10.1002/(SICI)1098-2396(199904)32:1<23::AID-SYN3>3.0.CO;2-N.