# CHARMM-GUI Ligand Binder for Absolute Binding Free Energy Calculations and Its Application

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**Table S1.** Anchoring atoms and reference restraint values used for FEP/MD simulations.

| Ligand | #[§] | $P_1$[¶] | $P_2$[¶] | $P_3$[¶] | $L_1$[¶] | $L_2$[¶] | $L_3$[¶] | $r_0$ | $\theta_0$ | $\phi_0$ | $\alpha_0$ | $\beta_0$ | $\gamma_0$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| benzene | C1 | 98 | 103 | 71 | C3 | C6 | C1 | 6.9 Å | 60.9˚ | 116.4˚ | 90.8˚ | -168.0˚ | -77.6˚ |
| | C2 | 98 | 95 | 150 | C3 | C6 | C2 | 6.8 Å | 82.3˚ | 97.7˚ | 97.2˚ | 77.0˚ | -99.6˚ |
| | C3 | 98 | 102 | 112 | C3 | C2 | C1 | 6.9 Å | 68.6˚ | -14.5˚ | 90.0˚ | -114.2˚ | 84.1˚ |
| indole | C1 | 98 | 94 | 3 | C8 | C1 | C5 | 6.8 Å | 107.6˚ | -136.2˚ | 65.0˚ | -138.2˚ | -79.7˚ |
| | C2 | 98 | 94 | 1 | C8 | C5 | C4 | 6.8 Å | 106.6˚ | -159.3˚ | 94.6˚ | 99.8˚ | -116.9˚ |
| | C3 | 98 | 94 | 152 | C8 | C7 | C3 | 6.9 Å | 105.2˚ | 103.1˚ | 64.9˚ | 165.2˚ | -107.4˚ |
| *n*-butylbenzene | C1 | 98 | 95 | 153 | C1 | C6 | C2 | 6.6 Å | 88.6˚ | 89.0˚ | 102.0˚ | 18.4˚ | 100.2˚ |
| | C2 | 98 | 93 | 2 | C1 | C2 | C4 | 6.6 Å | 104.4˚ | -152.6˚ | 109.6˚ | 65.7˚ | -98.4˚ |
| | C3 | 98 | 150 | 129 | C1 | C8 | C9 | 6.7 Å | 95.2˚ | -17.1˚ | 102.3˚ | -27.8˚ | -124.8˚ |
| SB3 | C1 | 59 | 51 | 54 | O2 | C5 | O4 | 9.3 Å | 88.8˚ | -0.05˚ | 71.7˚ | 120.5˚ | 127.5˚ |
| | C2 | 59 | 51 | 48 | O2 | O3 | C9 | 9.2 Å | 89.1˚ | -60.0˚ | 93.3˚ | -177.8˚ | -166.2˚ |
| | C3 | 59 | 51 | 21 | O2 | C6 | C10 | 9.2 Å | 88.8˚ | -86.1˚ | 82.8˚ | 137.1˚ | 146.6˚ |
| SBX | C1 | 59 | 80 | 75 | O2 | C8 | C12 | 9.4 Å | 75.4˚ | 111.8˚ | 104.4˚ | -90.7˚ | 154˚ |
| | C2 | 59 | 57 | 55 | O2 | C4 | O3 | 9.4 Å | 78.0˚ | -31.5˚ | 56.5˚ | -163.7˚ | -109.8˚ |
| | C3 | 59 | 99 | 101 | O2 | C23 | C10 | 9.5 Å | 79.8˚ | 109.2˚ | 139.4˚ | -133.4˚ | 158.1˚ |
| FK5 | C1 | 59 | 74 | 28 | O1 | C28 | C44 | 9.6 Å | 99.9˚ | 13.9˚ | 108.9˚ | 116.5˚ | -35.5˚ |
| | C2 | 55 | 49 | 51 | C24 | C27 | O9 | 6.1 Å | 100.5˚ | 145.0˚ | 74.3˚ | -160.5˚ | 152.6˚ |
| | C3 | 59 | 57 | 54 | O1 | C5 | O4 | 9.5 Å | 78.2˚ | -40.04˚ | 65.7˚ | -162.2˚ | 123.6˚ |

[§]C1, C2, and C3 represent independent FEP/MD simulations for each ligand. [¶]$P_1$, $P_2$, and $P_3$ are defined as the protein backbone atoms of the corresponding residue numbers in the PDB file. $L_1$, $L_2$, and $L_3$ are defined as the non-hydrogen ligand atoms that are bonded to the corresponding atoms. See Figure S1 for the ligand structures and the corresponding atom names.

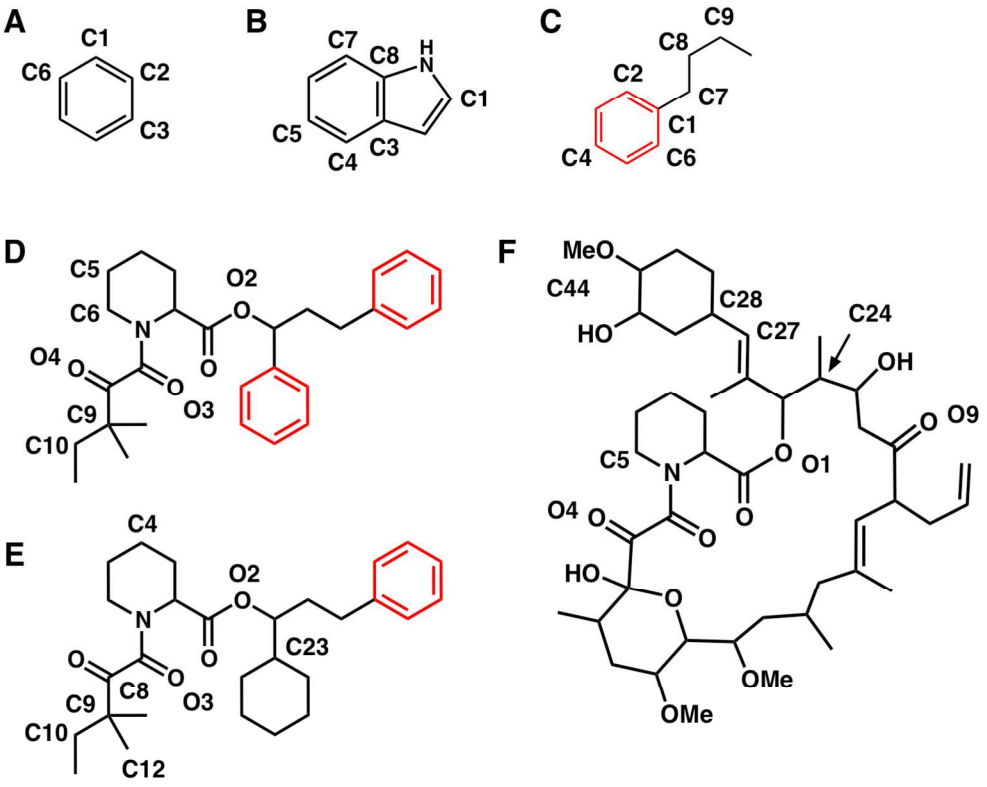


**Figure S1.** Structures of ligand molecules used in this work. Anchoring atoms used in the FEP/MD simulations are labeled. (A) Benzene, (B) Indole, (C) *n*-butylbenzene, (D) 1,3-diphenyl-1-propyl-1-(3,3-dimethyl-1,2-dioxypentyl)-2-piperidine carboxylate (SB3), (E) 1-cyclohexyl-3-diphenyl-1-propyl-1-(3,3-dimethyl-1,2-dioxypentyl)-2-piperidine carboxylate (SBX), and (F) K506 (FK5). Symmetric groups detected by *Ligand Binder* are colored in red.