***Supplementary material for***

**Exploring the unbinding mechanism of drugs from SERT via molecular dynamics simulation and its implication in antidepressants**

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**Table S1.** Hydrogen bond analysis with the equilibrium trajectories of the SERT- serotonin at S1 site complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acceptor** | **Donor** | **Occupancy**  **(%)** | **Average Distance(**Å**)** | **Average**  **Angle(°)** |
| SRO\_s1@N2 | 438SER@OG | 82.45 | 2.71 | 166.42 |
| SRO\_s1@O1 | 497THR@OG1 | 21.55 | 2.79 | 163.22 |
| 95TYR@OH | SRO\_s1@N1 | 15.67 | 2.91 | 159.19 |
| 97VAL@O | SRO\_s1@N2 | 14.79 | 2.90 | 158.94 |
| SRO\_s1@O1 | SRO\_s2@N2 | 13.11 | 2.89 | 154.35 |
| SRO\_s1@O1 | 175TYR@OH | 6.52 | 2.80 | 153.39 |
| 336SER@O | SRO\_s1@N1 | 6.51 | 2.88 | 152.86 |
| 335PHE@O | SRO\_s1@N1 | 4.56 | 2.84 | 150.32 |
| SRO\_s1@O1 | SRO\_s2@N2 | 3.14 | 2.88 | 154.32 |
| 438SER@OG | SRO\_s1@N2 | 0.97 | 2.90 | 151.94 |
| 438SER@O | SRO\_s1@N2 | 0.82 | 2.90 | 158.90 |
| 95TYR@O | SRO\_s1@N2 | 0.41 | 2.88 | 143.11 |
| 97VAL@O | SRO\_s1@N2 | 0.36 | 2.87 | 160.77 |
| 95TYR@O | SRO\_s1@N1 | 0.32 | 2.86 | 142.90 |
| 176TYR@OH | SRO\_s1@N2 | 0.12 | 2.90 | 153.71 |
| 438SER@OG | SRO\_s1@N2 | 0.11 | 2.89 | 154.45 |
| 98ASP@OD2 | SRO\_s1@N1 | 0.02 | 2.77 | 154.50 |
| 438SER@O | SRO\_s1@N2 | 0.02 | 2.92 | 157.22 |
| 98ASP@OD2 | SRO\_s1@N2 | 0.01 | 2.78 | 138.34 |
| SRO\_s1@N2 | 439THR@OG1 | 0.01 | 2.84 | 154.93 |
| 336SER@O | SRO\_s1@N2 | 0.01 | 2.93 | 159.12 |

**Table S2.** Hydrogen bond analysis with the equilibrium trajectories of the SERT- serotonin at S2 site complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acceptor** | **Donor** | **Occupancy**  **(%)** | **Average Distance(**Å**)** | **Average**  **Angle(°)** |
| SRO\_s2@N2 | 497THR@OG1 | 33.82 | 2.76 | 165.20 |
| SRO\_s2@O1 | 104ARG@NH2 | 25.63 | 2.86 | 151.13 |
| 494GLU@O | SRO\_s2@N1 | 17.97 | 2.86 | 157.76 |
| SRO\_s1@O1 | SRO\_s2@N2 | 13.11 | 2.89 | 154.35 |
| 98ASP@O1 | SRO\_s2@N2 | 9.22 | 2.83 | 159.59 |
| 493GLU@OE1 | SRO\_s2@N2 | 3.57 | 2.86 | 158.11 |
| 493GLU@O | SRO\_s2@N2 | 3.41 | 2.87 | 150.61 |
| SRO\_s2@N2 | 175TYR@OH | 3.39 | 2.80 | 158.24 |
| SRO\_s1@O1 | SRO\_s2@N2 | 3.14 | 2.88 | 154.32 |
| SRO\_s2@O1 | 104ARG@NE | 1.92 | 2.87 | 146.38 |
| 175TYR@OH | SRO\_s2@N2 | 1.54 | 2.91 | 161.87 |
| 497THR@OG1 | SRO\_s2@N2 | 0.45 | 2.88 | 147.00 |
| 493GLN@OE1 | SRO\_s2@N2 | 0.21 | 2.90 | 153.74 |
| 497THR@OG1 | SRO\_s2@N2 | 0.14 | 2.92 | 162.26 |
| 494ARG@OE2 | SRO\_s2@N1 | 0.13 | 2.83 | 153.69 |
| SRO\_s2@O1 | 104ARG@NH1 | 0.12 | 2.89 | 150.38 |
| 98ASP@OD2 | SRO\_s2@N2 | 0.09 | 2.91 | 162.64 |
| 493GLU@O | SRO\_s2@N2 | 0.07 | 2.87 | 148.39 |
| 175TYR@OH | SRO\_s2@N2 | 0.06 | 2.94 | 156.14 |
| SRO\_s2@O1 | 332GLN@NE2 | 0.02 | 2.94 | 153.25 |
| 494GLU@OE1 | SRO\_s2@O1 | 0.02 | 2.96 | 138.07 |
| 492LEU@O | SRO\_s2@N2 | 0.01 | 2.86 | 153.94 |
| 335PHE@O | SRO\_s2@N2 | 0.01 | 2.96 | 148.44 |
| 332GLN@NE2 | SRO\_s2@O1 | 0.01 | 2.98 | 135.10 |

**Table S3.** Hydrogen bond analysis with the equilibrium trajectories of the SERT- cocaine at S1 site complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acceptor** | **Donor** | **Occupancy**  **(%)** | **Average Distance(**Å**)** | **Average**  **Angle(°)** |
| COC\_s1@O4 | 176TYR@OH | 52.65 | 2.74 | 161.46 |
| COC\_s1@O4 | 438SER@OG | 11.57 | 2.75 | 160.61 |
| COC\_s1@O2 | 176TYR@OH | 8.55 | 2.80 | 162.59 |
| COC\_s1@O2 | 438SER@OG | 0.28 | 2.85 | 157.49 |
| COC\_s1@O1 | 96TYR@OH | 0.19 | 2.86 | 153.41 |
| COC\_s1@O2 | 96TYR@OH | 0.09 | 2.73 | 154.79 |
| COC\_s1@O4 | 439THR@OG1 | 0.01 | 2.75 | 141.88 |

**Table S4.** Hydrogen bond analysis with the equilibrium trajectories of the SERT- cocaine at S2 site complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acceptor** | **Donor** | **Occupancy**  **(%)** | **Average Distance(**Å**)** | **Average**  **Angle(°)** |
| COC\_s2@O2 | 104AGR@NH1 | 7.10 | 2.85 | 154.12 |
| COC\_s2@O2 | 104AGR@NE | 0.27 | 2.91 | 147.75 |
| COC\_s2@O4 | 104AGR@NH11 | 0.01 | 2.87 | 140.70 |
| COC\_s2@O4 | 104AGR@NE | 0.01 | 2.91 | 140.09 |

**Table S5.** Hydrogen bond analysis with the equilibrium trajectories of the SERT- escitalopram at S1 site complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acceptor** | **Donor** | **Occupancy**  **(%)** | **Average Distance(**Å**)** | **Average**  **Angle(°)** |
| 68P\_s1@N2 | 438SER@OG | 78.36 | 2.86 | 156.74 |
| 68P\_s1@F1 | 104ARG@NH2 | 6.12 | 3.01 | 132.08 |
| 68P\_s1@F1 | 104ARG@NH1 | 0.09 | 3.20 | 122.55 |
| 68P\_s1@F1 | 104ARG@NH2 | 0.06 | 3.06 | 127.50 |
| 68P\_s1@N2 | 176TYR@OH | 0.02 | 3.28 | 134.00 |

**Table S6.** Hydrogen bond analysis with the equilibrium trajectories of the SERT-escitalopram at S2 site complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acceptor** | **Donor** | **Occupancy**  **(%)** | **Average Distance(**Å**)** | **Average**  **Angle(°)** |
| 68P\_s2@N2 | 104ARG@NE | 55.00 | 3.26 | 159.56 |
| 68P\_s2@N2 | 104ARG@NH1 | 9.77 | 3.25 | 144.23 |
| 68P\_s2@F1 | 498GLY@N | 0.52 | 3.40 | 123.85 |
| 68P\_s2@N2 | 107TYR@OH | 0.06 | 3.10 | 163.02 |
| 68P\_s2@N1 | 559SER@OG | 0.01 | 3.38 | 126.41 |
| 68P\_s2@N2 | 562GLN@NE2 | 0.01 | 3.44 | 158.11 |

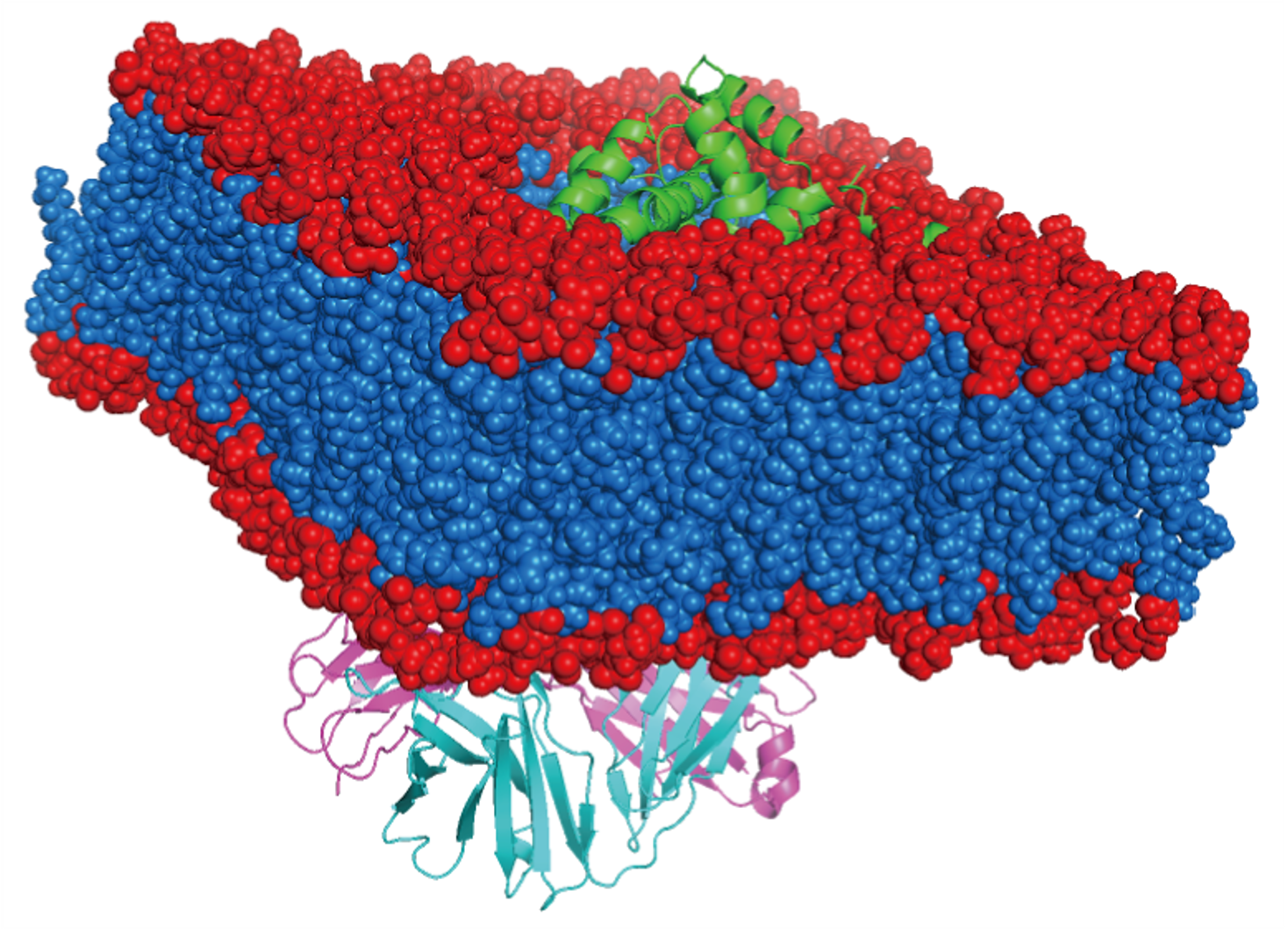
**Table S7.** Hydrogen bond analysis with the equilibrium trajectories of the SERT-paroxetine at S1 site complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acceptor** | **Donor** | **Occupancy**  **(%)** | **Average Distance(**Å**)** | **Average**  **Angle(°)** |
| 95TYR@O | 8PR\_s1@N1 | 99.52 | 2.91 | 161.93 |
| 8PR\_s1@O | 175TYR@OH | 8.43 | 3.17 | 132.33 |
| 336SER@O | 8PR\_s1@N1 | 1.41 | 3.36 | 126.37 |
| 8PR\_s1@F1 | 498GLY@N | 0.75 | 3.21 | 125.58 |
| 8PR\_s1@F1 | 497THR@OG1 | 0.70 | 2.95 | 150.25 |
| 8PR\_s1@O1 | 438SER@OG | 0.32 | 3.34 | 126.84 |
| 94GLY@O | 8PR\_s1@N1 | 0.03 | 3.29 | 132.59 |
| 8PR\_s1@N1 | 98ASP@N | 0.03 | 3.40 | 122.96 |
| 338GLY@N | 8PR\_s1@N1 | 0.02 | 3.35 | 145.49 |
| 337LEU@N | 8PR\_s1@N1 | 0.01 | 3.45 | 125.13 |

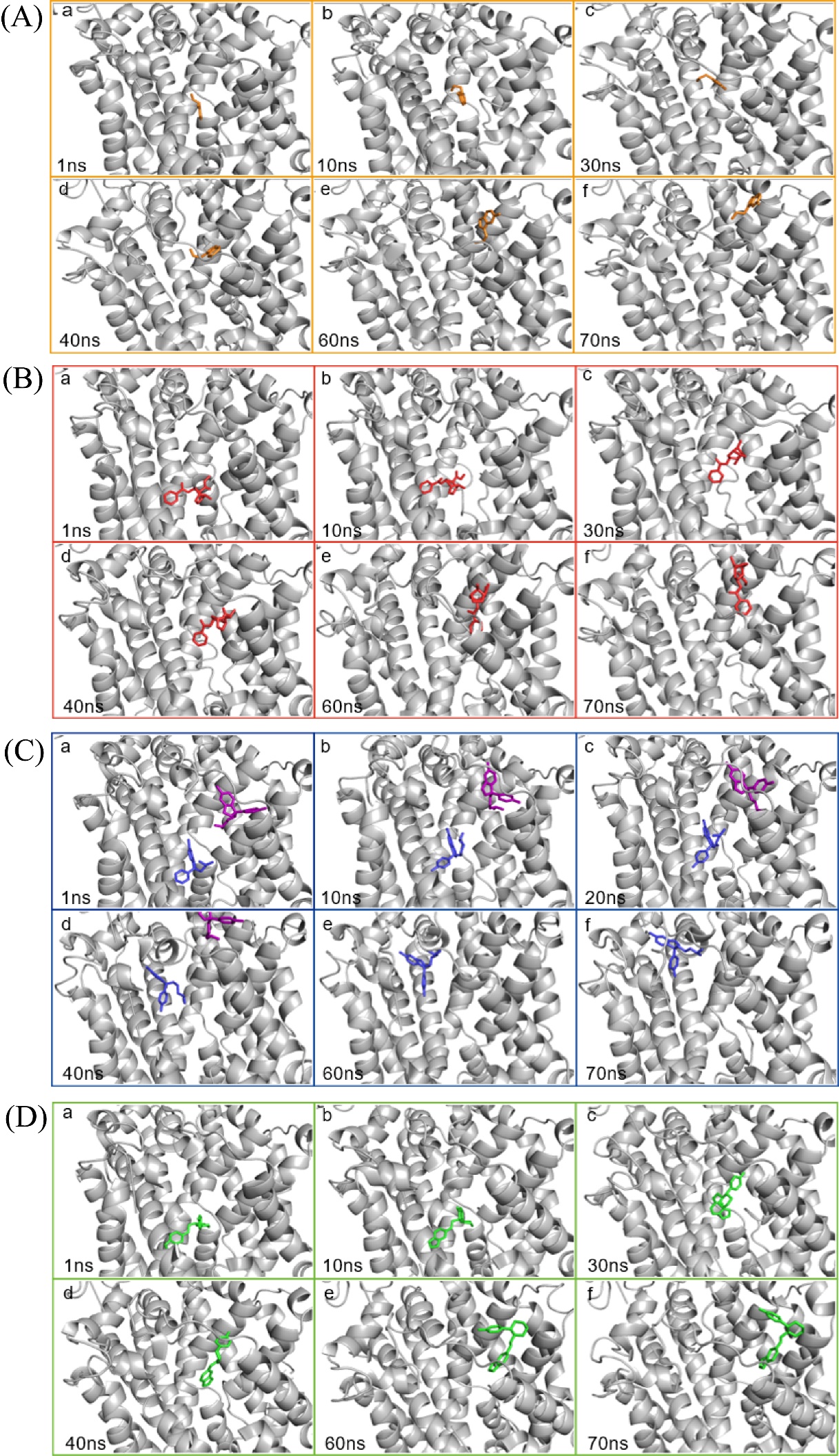
**Table S8.** The energy component of binding free energy (MM/GBSA method).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| complex | ΔEvdW | ΔEele | ΔGpol, GB | ΔGnonpol | ΔGgas | ΔGsol | ΔGtotal |
| SERT-serotonin\_s1a | -29.26c | -17.80 | 22.15 | -3.47 | -47.06 | 18.68 | -28.38 |
| hSET-cocaine\_s1 | -42.53 | -1.71 | 2.93 | -5.11 | -44.24 | -2.18 | -46.42 |
| SERT-escitalopram\_s1 | -43.67 | -66.25 | 64.53 | -6.07 | -109.93 | 58.46 | -51.47 |
| SERT-paroextine\_s1 | -50.63 | -7.47 | 12.68 | -5.92 | -58.10 | 6.76 | -51.34 |
| SERT-serotonin\_s2b | -24.00 | -13.59 | 21.87 | -3.16 | -37.59 | 18.71 | -18.88 |
| SERT-cocaine\_s2 | -39.47 | -0.45 | 5.12 | -4.80 | -39.92 | 0.32 | -39.60 |
| SERT-escitalopram\_s2 | -39.55 | -63.00 | 69.38 | -5.48 | -102.55 | 64.80 | -38.76 |

aSubstrate/Drug binding to the orthosteric (S1) site of SERT. bSubstrate/Drug binding to the allosteric (S2) site of SERT. cAll values are in kcal/mol



**Figure S1**. The model of the membrane protein of SERT. the protein is inserted into DOPC lipid bilayer used by CHARMM-GUI. lipid bilayer are colored in red and blue with spheres representation. sodium-dependent serotonin transporter and two 8B6 antibody molecules are colored in green, cyan, and magenta in cartoon representations respectively.

**Figure S2.** The intermediate snapshots of four system during the PMF simulation, SERT and ligands were represented as gray cartoon and colored sticks, respectively. Only the escitalopram of at S1 site pulled out the channel directly along MT6, other six molecules moved towards between MT1b and MT6a. (A) The SERT-serotonin system. The serotonin at S1 site, colored in orange, dissociated from SERT moving towards between MT1b and MT6a similar to the one at S2 site. (B)The SERT-cocaine system. the cocaine at S1 site, colored in red, dissociated from SERT moving towards between MT1b and MT6a similar to the one at S2 site. (C) The SERT-escitalopram system. The escitalopram (colored in blue) at S1 site pulled out the channel directly along MT6 and the one (colored in purple) at S2 site moved toward between MT1b and MT6a. (D) The SERT-paroxetine system. the paroxetine at S1 site, colored in green, dissociated from SERT moving towards between MT1b and MT6a similar to the one at S2 site.