Supporting Information for:

Regulation of Cholesterol Binding to the Receptor Patched1 by its interactions With the Ligand Sonic Hedgehog (Shh)

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Table S1. The Lipid compositions of the outer and inner leaflets of our membrane model.

| Type            | Outer leaflet | Inner leaflet |
|-----------------|---------------|---------------|
|                 | No. | Percentage | No.   | Percentage |
| CHOL            | 206 | 32.85%     | CHOL  | 180 | 29.22%     |
| DAPC di-C20:4-C22:5 PC | 2   | 0.32%      | DAPC  | 1   | 0.16%      |
| DOPC di-C16:1-C18:1 PC | 6   | 0.96%      | DOPC  | 3   | 0.49%      |
| PAPC C16:0/20:4 PC | 18  | 2.87%      | PAPC  | 9   | 1.46%      |
| PEPC C16:0/20:2 PC | 4   | 0.64%      | PEPC  | 2   | 0.32%      |
| PIPC C16:0/18:2 PC | 113 | 18.02%     | PIPC  | 57  | 9.25%      |
| POPC C16:0/18:1 PC | 78  | 12.44%     | POPC  | 39  | 6.33%      |
| PUPC C16:0/22:6 PC | 4   | 0.64%      | PUPC  | 2   | 0.32%      |
| DAPE di-C20:4-C22:5 PE | 5   | 0.80%      | DAPE  | 23  | 3.73%      |
| DOPE di-C16:1-C18:1 PE | 2   | 0.32%      | DOPE  | 13  | 2.11%      |
| DUPE di-C20:5-C22:6 PE | 1   | 0.16%      | DUPE  | 6   | 0.97%      |
| PAPE C16:0/20:4 PE | 8   | 1.28%      | PAPE  | 37  | 6.01%      |
| PIPE C16:0/18:2 PE | 5   | 0.80%      | PIPE  | 27  | 4.38%      |
| POPE C16:0/18:1 PE | 8   | 1.28%      | POPE  | 39  | 6.33%      |
| PQPE C16:0/20:3 PE | 1   | 0.16%      | PQPE  | 6   | 0.97%      |
| PUPE C16:0/22:6 PE | 2   | 0.32%      | PUPE  | 13  | 2.11%      |
| BNSM C(d20:1/24:1) SM | 12  | 1.91%      | BNSM  | 6   | 0.97%      |
| DBSM C(d20:1/20:0) SM | 8   | 1.28%      | DBSM  | 4   | 0.65%      |
| DPSM C(d18:1/18:0) SM | 40  | 6.38%      | DPSM  | 19  | 3.08%      |
| DXSM C(d24:1/24:0) SM | 16  | 2.55%      | DXSM  | 8   | 1.30%      |
| PGSM C(d18:1/22:1) SM | 2   | 0.32%      | PGSM  | 1   | 0.16%      |
| PNSM C(d18:1/24:1) SM | 25  | 3.99%      | PNSM  | 11  | 1.79%      |
| POSM C(d18:1/18:1) SM | 2   | 0.32%      | POSM  | 1   | 0.16%      |
| XNSM C(d24:1/24:1) SM | 17  | 2.71%      | XNSM  | 8   | 1.30%      |
| DPG1 C(d18:1/18:0) GM1 | 16  | 2.55%      | DPG1  | 1   | 0.16%      |
| DPG3 C(d18:1/18:0) GM3 | 15  | 2.39%      | DPG3  | 1   | 0.16%      |
| DPCE C(d18:1/18:0) CER | 2   | 0.32%      | DPCE  | 31  | 5.03%      |
| DXCE C(d24:1/24:0) CER | 1   | 0.16%      | DXCE  | 4   | 0.65%      |
| PNC C(d18:1/24:1) CER | 1   | 0.16%      | PNC   | 14  | 2.27%      |
| XNCE C(d24:1/24:1) CER | 1   | 0.16%      | XNCE  | 2   | 0.32%      |
| PODG C16:0/18:1 DAG | 1   | 0.16%      | PODG  | 12  | 1.95%      |
| PIDG C16:0/18:2 DAG | 1   | 0.16%      | PIDG  | 7   | 1.14%      |
| PADG C16:0/20:4 DAG | 4   | 0.64%      | PADG  | 8   | 1.30%      |
| POPI C16:0/18:1 PI | 9   | 1.46%      | POPI  | 3   | 0.49%      |
| PUPI C16:0/22:6 PI | 3   | 0.49%      | PUPI  | 2   | 0.32%      |
| POPA C16:0/18:1 PA | 3   | 0.49%      | POPA  | 2   | 0.32%      |
| PIPA C16:0/18:2 PA | 2   | 0.32%      | PIPA  | 1   | 0.16%      |
| PUPA C16:0/22:6 PA | 1   | 0.16%      | PUPA  | 1   | 0.22%      |
| DPCE   | C(d18:1/18:0) CER | 1 | 0.16% |
|--------|------------------|---|------|
| PODG   | C16:0/18:1 DAG   | 1 | 0.16% |
| PIDG   | C16:0/18:2 DAG   | 1 | 0.16% |
| PADG   | C16:0/20:4 DAG   | 1 | 0.16% |
| cut-off(nm) | PC | PE | CHOL | SM |
|------------|----|----|------|-----|
|            | 0.7 | 1.4 | 2.1 | 0.7 | 1.4 | 2.1 | 0.7 | 1.4 | 2.1 | 0.7 | 1.4 | 2.1 |
| **Monomer** | 0.34±0.14 | 0.85±0.19 | 0.95±0.16 | 1.64±0.27 | 1.22±0.29 | 0.95±0.16 | 1.02±0.10 | 0.88±0.13 | 0.95±0.11 | 0.15±0.12 | 0.69±0.27 | 0.91±0.24 |
|            | 0.49±0.12 | 0.74±0.18 | 0.88±0.18 | 1.49±0.25 | 1.33±0.33 | 0.88±0.18 | 0.91±0.10 | 0.84±0.14 | 0.96±0.13 | 0.11±0.11 | 0.63±0.25 | 0.87±0.26 |
|            | 0.45±0.11 | 0.83±0.16 | 0.98±0.15 | 1.35±0.22 | 1.19±0.32 | 0.98±0.15 | 1.12±0.11 | 0.84±0.12 | 0.95±0.12 | 0.17±0.11 | 0.71±0.25 | 0.86±0.23 |
|            | 0.53±0.11 | 0.81±0.14 | 0.99±0.15 | 1.37±0.26 | 1.21±0.22 | 0.99±0.15 | 0.98±0.12 | 0.84±0.13 | 0.99±0.11 | 0.32±0.15 | 0.61±0.23 | 1.03±0.26 |
|            | 0.61±0.13 | 0.76±0.14 | 0.85±0.15 | 1.47±0.28 | 1.22±0.27 | 0.85±0.15 | 0.98±0.11 | 0.84±0.13 | 0.93±0.11 | 0.16±0.13 | 0.68±0.21 | 0.87±0.23 |
| **Dimer-Shh** | 0.61±0.09 | 0.81±0.14 | 1.09±0.14 | 1.56±0.15 | 1.17±0.20 | 1.09±0.14 | 0.90±0.07 | 0.89±0.11 | 0.93±0.10 | 0.15±0.09 | 0.70±0.19 | 1.02±0.20 |
|            | 0.62±0.09 | 0.78±0.13 | 0.97±0.12 | 1.41±0.20 | 1.07±0.19 | 0.97±0.12 | 1.07±0.07 | 0.87±0.09 | 0.99±0.11 | 0.25±0.10 | 0.83±0.18 | 1.23±0.19 |
|            | 0.51±0.10 | 0.98±0.13 | 1.06±0.13 | 1.39±0.22 | 1.13±0.20 | 1.06±0.13 | 0.99±0.08 | 0.88±0.10 | 0.96±0.10 | 0.20±0.09 | 0.69±0.18 | 0.99±0.20 |
|            | 0.58±0.09 | 0.76±0.14 | 0.97±0.16 | 1.35±0.17 | 1.07±0.21 | 0.97±0.16 | 1.10±0.07 | 0.88±0.10 | 0.95±0.10 | 0.19±0.08 | 0.83±0.17 | 1.08±0.21 |
|            | 0.57±0.10 | 0.83±0.13 | 1.03±0.13 | 1.27±0.19 | 1.03±0.21 | 1.03±0.13 | 1.01±0.09 | 0.91±0.10 | 0.95±0.09 | 0.42±0.12 | 0.82±0.16 | 1.07±0.20 |
| **cut-off(nm)** | GM | PS | PI | PA |
|            | 0.7 | 1.4 | 2.1 | 0.7 | 1.4 | 2.1 | 0.7 | 1.4 | 2.1 | 0.7 | 1.4 | 2.1 |
| **Monomer** | 4.08±0.83 | 3.62±1.02 | 1.74±0.66 | 1.88±0.57 | 1.37±0.58 | 0.98±0.39 | 3.26±1.07 | 1.04±0.83 | 0.85±0.72 | 2.01±2.37 | 1.38±1.50 | 1.35±1.48 |
|            | 4.21±0.68 | 4.32±0.83 | 2.18±0.70 | 2.15±0.55 | 1.33±0.58 | 1.06±0.49 | 2.67±1.17 | 1.04±1.00 | 0.92±0.74 | 1.35±1.70 | 1.12±1.45 | 0.92±1.32 |
|            | 3.87±0.77 | 4.75±0.96 | 2.52±0.56 | 1.02±0.40 | 1.11±0.59 | 1.02±0.49 | 3.44±1.25 | 1.47±0.91 | 1.06±0.66 | 5.87±1.68 | 0.66±1.31 | 0.66±1.18 |
|            | 2.82±0.58 | 5.74±0.93 | 1.50±0.57 | 1.47±0.76 | 1.05±0.53 | 0.94±0.45 | 4.15±1.54 | 1.06±0.83 | 0.90±0.65 | 1.45±1.75 | 0.72±1.12 | 0.86±1.41 |
|            | 2.73±0.57 | 5.53±0.81 | 4.46±0.66 | 1.46±0.48 | 1.05±0.47 | 0.91±0.41 | 3.41±1.16 | 1.01±0.75 | 0.67±0.58 | 1.34±1.56 | 1.00±1.74 | 0.85±1.13 |
| **Dimer-Shh** | 4.02±0.47 | 5.20±0.63 | 1.16±0.44 | 1.33±0.37 | 1.00±0.45 | 0.95±0.38 | 3.97±0.74 | 0.92±0.59 | 0.58±0.40 | 1.51±1.45 | 0.86±1.17 | 0.93±0.99 |
|            | 2.57±0.38 | 5.16±0.53 | 1.15±0.30 | 1.76±0.45 | 1.05±0.35 | 0.98±0.31 | 2.51±0.65 | 0.89±0.63 | 0.70±0.48 | 2.56±2.18 | 0.78±0.95 | 0.73±0.92 |
|            | 3.56±0.40 | 2.80±0.52 | 1.08±0.41 | 2.12±0.37 | 1.18±0.39 | 1.05±0.37 | 3.55±0.61 | 1.39±0.69 | 0.80±0.51 | 1.81±1.19 | 1.27±1.37 | 0.90±1.05 |
|            | 2.25±0.29 | 5.62±0.51 | 2.11±0.32 | 1.39±0.30 | 1.01±0.32 | 0.93±0.32 | 3.95±0.72 | 0.97±0.57 | 0.73±0.46 | 3.78±1.15 | 0.85±1.05 | 0.61±0.92 |
|            | 3.10±0.41 | 4.48±0.61 | 1.29±0.58 | 1.89±0.33 | 0.97±0.34 | 0.87±0.30 | 2.51±0.80 | 1.15±0.72 | 0.78±0.55 | 3.08±1.65 | 1.65±1.37 | 1.16±1.12 |
Table S3. The D-E index of lipids with different unsaturation within 0.7nm, 1.4nm and 2.1nm of proteins.

| No. unsaturated bonds in two hydrophobic tail (the content) | Distance (nm) | PTCH1-monomer | PTCH1-dimer |
|-----------------------------------------------------------|---------------|----------------|-------------|
| 0/0 (8.93%) | 0.7 | 0.59±0.23 0.80±0.19 0.44±0.19 0.60±0.16 0.67±0.21 | 0.51±0.15 0.35±0.12 0.64±0.11 0.59±0.12 0.69±0.19 |
| | 1.4 | 1.35±0.32 1.35±0.41 1.12±0.37 1.50±0.30 1.37±0.36 | 1.35±0.23 1.55±0.25 1.00±0.25 1.48±0.20 1.45±0.25 |
| | 2.1 | 1.04±0.31 1.07±0.39 1.30±0.29 0.99±0.28 1.74±0.30 | 0.93±0.24 1.26±0.29 1.00±0.23 1.24±0.24 1.10±0.28 |
| 1/0 (23.33%) | 0.7 | 0.49±0.11 0.43±0.12 0.73±0.13 0.62±0.12 0.29±0.11 | 0.56±0.08 0.59±0.10 0.59±0.10 0.44±0.08 0.55±0.12 |
| | 1.4 | 0.81±0.18 0.84±0.20 1.06±0.21 0.89±0.21 0.89±0.19 | 0.94±0.16 1.01±0.13 0.95±0.16 1.02±0.14 0.90±0.16 |
| | 2.1 | 0.97±0.19 0.95±0.18 0.87±0.21 1.07±0.18 0.88±0.17 | 1.05±0.14 1.05±0.16 0.98±0.16 1.07±0.16 1.00±0.15 |
| 1/1 (1.93%) | 0.7 | 0.67±0.75 0.45±0.60 1.04±0.53 0.17±0.38 0.37±0.58 | 0.44±0.42 0.51±0.49 0.96±0.45 0.47±0.40 0.67±0.47 |
| | 1.4 | 1.15±1.08 0.84±0.74 0.67±0.76 1.15±0.72 0.74±0.82 | 0.87±0.59 0.49±0.47 0.79±0.62 0.73±0.56 0.96±0.59 |
| | 2.1 | 1.12±0.90 0.90±0.72 0.69±0.59 0.97±0.70 0.87±0.61 | 1.16±0.59 0.67±0.52 1.12±0.68 1.01±0.48 1.03±0.63 |
| 2/0 (17.38%) | 0.7 | 0.60±0.17 0.53±0.19 0.63±0.18 0.64±0.18 0.99±0.20 | 0.69±0.14 0.79±0.12 0.69±0.17 0.95±0.16 0.71±0.18 |
| | 1.4 | 0.94±0.25 0.81±0.21 0.88±0.24 0.93±0.23 0.90±0.21 | 0.95±0.17 0.87±0.19 1.05±0.21 0.87±0.24 0.97±0.21 |
| | 2.1 | 1.01±0.22 0.93±0.21 0.99±0.23 0.98±0.21 0.91±0.20 | 1.11±0.20 0.95±0.18 1.05±0.18 0.95±0.20 1.10±0.20 |
| 4/0 (9.01%) | 0.7 | 1.36±0.35 1.66±0.46 1.37±0.35 1.39±0.42 1.26±0.34 | 2.00±0.30 1.48±0.24 1.41±0.32 1.21±0.28 1.65±0.21 |
| | 1.4 | 1.17±0.42 1.36±0.47 1.25±0.43 1.11±0.40 1.23±0.36 | 1.11±0.28 0.95±0.30 1.16±0.30 1.04±0.26 0.87±0.28 |
| | 2.1 | 1.15±0.36 1.19±0.39 1.03±0.36 1.03±0.30 1.03±0.33 | 0.99±0.25 1.00±0.26 1.11±0.26 0.98±0.27 0.97±0.28 |
| 4/4 (2.50%) | 0.7 | 3.75±1.48 4.83±1.76 2.49±1.30 3.13±1.27 3.96±1.57 | 4.40±0.61 3.43±0.73 3.07±0.96 2.91±0.63 2.58±0.88 |
| | 1.4 | 1.50±1.03 2.15±1.03 1.78±1.04 1.74±1.05 2.01±1.14 | 1.49±0.71 1.09±0.62 1.54±0.72 1.35±0.71 1.51±0.77 |
| | 2.1 | 0.96±0.67 1.43±0.81 1.45±0.90 0.72±0.62 1.10±0.80 | 0.86±0.45 0.67±0.51 0.86±0.58 0.88±0.47 1.00±0.63 |
| 5/0 (2.98%) | 0.7 | 2.77±0.94 1.77±1.08 1.83±0.68 2.67±0.87 1.71±0.70 | 2.10±0.53 2.52±0.51 2.52±0.66 2.81±0.58 2.24±0.63 |
| | 1.4 | 1.62±0.82 1.15±0.63 1.25±0.76 1.53±0.96 1.18±0.66 | 1.16±0.51 1.42±0.50 1.53±0.61 1.12±0.53 1.12±0.60 |
| | 2.1 | 1.11±0.69 1.03±0.70 1.21±0.59 0.94±0.61 1.04±0.67 | 1.14±0.55 0.92±0.43 1.07±0.51 0.84±0.46 1.01±0.47 |
Figure S1. The detailed structures of the SSD and SSDL pockets and their states of CHOL binding in the structures of experimental reports. The coloring strategy is the same as Figure 1, ECD1 in light blue, ECD2 in yellow, Shh in red, SSD in magenta, and SSDL in purple. CHOLs are shown in red sticks. The labels are their PDB ID.
Figure S2. The detailed structure of the dimer in the lipid bilayer. The coloring strategy is the same as Figure S1.
Figure S3. Center-of-mass distances between SSDL P1082 and the nearest CHOL ROH bead in each simulation of PTCH1 monomer, dimer-Shh PTCH1-A and PTCH1-B.
Figure S4. The populations of the number of residues interacting with CHOLs in all simulations of the monomer and dimer-Shh (PTCH1-A and PTCH1-B).
Figure S5. The subsites A and B of CHOL binding.
Figure S6. Convergence of the cumulative average of the number of lipids around PTCH1 in the simulations of the monomer (top) and dimer-Shh (PTCH1-A:middle, PTCH1-B: bottom) systems.
Figure S7. The hotspots maps of residues involved in lipid-PTCH1 interactions, showing the SSD (A) and SSDL sides (B).
Figure S8. Root mean square deviations (RMSDs) of backbones of PTCH1-B, superimposed on backbones of the initial structure of PTCH1-A.
Figure S9. Dynamical cross-correlation matrices for BB beads for (A) PTCH1 monomer and (B) dimer-Shh, using 10, 20 and 30 μs of the simulation trajectories. The DCCMs after 30μs are well converged.
Figure S10. The optimal pathways of Y446-L248.
Figure S11. The optimal pathways of Y446-Y379.
Figure S12. The optimal pathways of Y446-M956.