Table S1. MM/GBSA docking scores of intermediates I1 and I2 in reaction channels A, B, and C.

Table S1a. Induced fit docking of intermediates against crystal structures (using QM chargesa for the ligands)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PDB | A-I1 | B-I1 | C-I1 | D-I1 | A-I2 | B-I2 | C-I2 | D-I2 |
| 1SQC | **-57.8b** | n.p.c | -41.9 | n.p. | -33.2 | n.p. | -6.0 | n.p. |
| 1W6K | -62.7 | -62.3 | **-73.5** | -59.1 | -35.0 | -32.8 | -43.3 | -46.3 |

aQM charges are electrostatic potential atomic charges derived at the HF/6-31G\* level.

bbolded numbers are the 'decision-making' intermediates

cn.p. means no pose can be generated

Table S1b. Induced fit docking of intermediates against crystal structures (using MM chargesa for the ligands)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PDB | A-I1 | B-I1 | C-I1 | D-I1 | A-I2 | B-I2 | C-I2 | D-I2 |
| 1SQC | **-52.2** | n.p. | -47.6 | n.p. | -38.3 | n.p. | -26.8 | n.p. |
| 1W6K | -68.2 | -69.6 | **-72.8** | -66.3 | -38.1 | -46.7 | -44.1 | -54.8 |

aMM charges are OPLS2005 charges, generated by Schrödinger Ligprep

bbolded numbers are the 'decision-making' intermediates

cn.p. means no pose can be generated

Table S1c. Reaction channel predictions for triterpenoid synthases in the 1SQC cluster (yellow color indicates incorrect predictions; bolded numbers indicate the intermediates used to predict the reaction channel, using the procedure described in Methods)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| GI | Uniprot | SwissProt | EC number | common name | Sequence  identity to 1SQC | SwissProt /Predicted  Channel | A-I1 | B-I1 | C-I1 | D-I1 | A-I2 | B-I2 | C-I2 | D-I2 |
| 357580429 | B3Y522 | DCD\_DRYCA | 5.4.99.37 | dammara-20,24-diene | 42% | B/B | n.p. | **-42.0** | -27.9 | -31.6 | n.p.a | 1.9 | 4.3 | 18.2 |
| 6466213 | P33990 | SQHC\_ZYMMO | 4.2.1.129; 5.4.99.17 | hopanol; hop-22(29)-ene | 39% | A/A | **-40.0** | n.p. | -31.0 | -34.0 | -18.4 | n.p. | -19.2 | -24.2 |
| 16519641 | P55348 | SQHC\_RHISN | 4.2.1.129; 5.4.99.17 | hopanol; hop-22(29)-ene | 38% | A/A | **-41.4** | -38.4 | -33.4 | n.p. | -25.1 | -18.2 | 2.6 | n.p. |
| 2113823 | P54924 | SQHC\_BRAJA | 4.2.1.129; 5.4.99.17 | hopanol; hop-22(29)-ene | 38% | A/A | **-48.6** | -40.1 | -40.1 | -37.6 | -20.9 | -25.2 | -39.4 | -13.8 |

an.p. means no pose can be generated

Table S1d. Reaction channel predictions for triterpenoid synthases in the 1W6K cluster (yellow color indicates incorrect predictions; bolded numbers indicate the intermediates used to predict the reaction channel, using the procedure described in Methods)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| GI | Uniprot | SwissProt | EC number | common name | Sequence identity to 1W6K | SwissProt /Predicted Channel | A-I1 | B-I1 | C-I1 | D-I1 | A-I2 | B-I2 | C-I2 | D-I2 |
| 114053041 | P84466 | ERG7\_BOVIN | 5.4.99.7 | lanosterol | 86% | C/C | -67.8 | -61.3 | **-72.8** | -57.4 | -41.0 | -38.5 | -39.4 | -50.7 |
| 26346907 | Q8BLN5 | ERG7\_MOUSE | 5.4.99.7 | lanosterol | 86% | C/C | n.p. | -74.7 | **-75.8** | -55.4 | n.p. | -37.6 | -40.2 | -41.5 |
| 13591981 | P48450 | ERG7\_RAT | 5.4.99.7 | lanosterol | 85% | C/C | n.p. | -56.4 | **-76.6** | -66.4 | n.p. | -41.1 | -54.5 | -48.4 |
| 15076955 | Q96WJ0 | ERG7\_PNECA | 5.4.99.7 | lanosterol | 46% | C/C | -55.4 | -73.4 | **-77.8** | -66.0 | -16.8 | -38.5 | -34.0 | -46.7 |
| 167295241 | P38604 | ERG7\_YEAST | 5.4.99.7 | lanosterol | 41% | C/C | n.p. | -72.2 | **-78.9** | -66.5 | n.p. | -26.4 | -27.6 | -30.2 |
| 63054562 | Q10231 | ERG7\_SCHPO | 5.4.99.7 | lanosterol | 42% | C/C | 28.4 | -77.1 | **-84.9** | -58.8 | -48.2 | -43.6 | -50.8 | -38.8 |
| 68466833 | Q04782 | ERG7\_CANAL | 5.4.99.7 | lanosterol | 40% | C/C | -68.4 | n.p. | **-81.7** | -60.3 | -25.3 | n.p. | -39.7 | -41.3 |
| 66825783 | Q55D85 | CAS1\_DICDI | 5.4.99.8 | cycloartenol | 49% | C/C | -59.9 | -74.1 | **-78.2** | -61.2 | -46.4 | -47.3 | -39.7 | -30.5 |
| 300591899 | O82139 | CAS1\_PANGI | 5.4.99.8 | cycloartenol | 45% | C/C | -61.5 | n.p. | **-77.7** | -41.6 | -26.8 | n.p. | -34.3 | -23.9 |
| 300592019 | Q9SXV6 | CAS1\_GLYGL | 5.4.99.8 | cycloartenol | 45% | C/C | -73.0 | -79.5 | **-81.4** | -53.7 | -43.4 | -46.3 | -42.3 | -45.8 |
| 300591983 | Q8W3Z4 | CAS1\_BETPL | 5.4.99.8 | cycloartenol | 45% | C/C | -59.6 | n.p. | **-85.3** | -58.6 | -41.9 | n.p. | -46.2 | -38.2 |
| 300592007 | Q9SLP9 | CAS1\_LUFCY | 5.4.99.8 | cycloartenol | 45% | C/C | n.p. | -80.2 | **-81.5** | -65.8 | n.p. | -47.5 | -49.3 | -56.6 |
| 82468805 | Q2XPU6 | CAS1\_RICCO | 5.4.99.8 | cycloartenol | 44% | C/C | n.p. | n.p. | **-80.6** | -56.6 | n.p. | n.p. | -47.7 | -48.8 |
| 300591913 | Q6BE25 | CAS1\_CUCPE | 5.4.99.8 | cycloartenol | 44% | C/C | -70.3 | n.p. | **-85.8** | -67.0 | -47.4 | n.p. | -41.9 | -43.9 |
| 300591981 | Q8W3Z3 | CAS2\_BETPL | 5.4.99.8 | cycloartenol | 45% | C/C | n.p. | n.p. | **-85.8** | -60.9 | n.p. | n.p. | -46.4 | -34.3 |
| 6090879 | Q6Z2X6 | CAS\_ORYSJ | 5.4.99.8 | cycloartenol | 43% | C/C | -74.6 | n.p. | **-85.3** | -49.0 | -45.5 | n.p. | -43.4 | -41.3 |
| 119499584 | A1CVK0 | PDSA\_NEOFI | 5.4.99.32 | (17Z)-protosta-17(20),24-dien-3beta-ol | 40% | C/C | -69.1 | n.p. | **-73.0** | -66.8 | -41.4 | n.p. | -17.6 | -35.5 |
| 70993016 | B0Y5B4 | PDSA\_ASPFC | 5.4.99.32 | (17Z)-protosta-17(20),24-dien-3beta-ol | 40% | C/C | -67.7 | n.p. | **-73.8** | -58.7 | -23.7 | n.p. | -34.0 | -44.2 |
| 300591911 | Q6BE24 | CUCS\_CUCPE | 5.4.99.33 | cucurbitadienol | 43% | C/C | n.p. | -68.6 | **-73.2** | -46.0 | n.p. | -45.3 | -50.3 | -33.5 |
| 108864084 | H2KWF1 | PAKSY\_ORYSJ | 5.4.99.47 | parkeol | 39% | C/C | -48.9 | n.p. | **-70.8** | -48.4 | -34.0 | n.p. | -41.3 | -34.9 |
| 300807982 | E2IUB0 | CASS\_KALDA | 5.4.99.8 | cycloartenol | 44% | C/C | -77.9 | n.p. | **-82.7** | -57.3 | -51.0 | n.p. | -44.3 | 20.6 |
| 30699377 | Q8RWT0 | LUP2\_ARATH | 5.4.99.39; 5.4.99.40; 5.4.99.41 | beta-amyrin; alpha-amyrin; lupeol | 39% | B/B | -27.2 | **-58.5** | -51.0 | -26.1 | -22.9 | -30.2 | -42.3 | -30.0 |
| 353678016 | A8CDT2 | BAS\_BRUGY | 5.4.99.39 | beta-amyrin | 41% | B/B | -44.7 | **-51.0** | n.p. | -38.8 | -40.3 | -38.5 | n.p. | -33.5 |
| 118901781 | O82146 | BAMS2\_PANGI | 5.4.99.39 | beta-amyrin | 40% | B/B | -49.0 | **-54.7** | -51.8 | n.p. | -45.1 | -47.0 | -51.4 | n.p. |
| 350538403 | E7DN64 | DAMS\_SOLLC | 5.4.99.55 | delta-amyrin | 39% | B/B | -35.9 | **-74.4** | -66.8 | -59.2 | -38.0 | -37.8 | -54.2 | -44.9 |
| 300807980 | E2IUA9 | LUPS\_KALDA | 5.4.99.41 | lupeol | 39% | B/B | -40.6 | **-64.6** | n.p. | 46.6 | -50.4 | -51.8 | n.p. | -39.2 |
| 82468803 | Q2XPU7 | LUPS\_RICCO | 5.4.99.41 | lupeol | 37% | B/B | -46.1 | **-71.5** | -64.0 | -25.0 | -39.9 | -40.3 | -41.9 | -46.7 |
| 353678133 | A8C981 | TARS\_RHISY | 5.4.99.35 | taraxerol | 37% | B/B | -58.6 | **-63.8** | n.p. | -43.1 | -33.0 | -30.2 | n.p. | -34.9 |
| 240256372 | Q9LVY2 | PEN3\_ARATH | 5.4.99.56 | tirucalla-7,24-dien-3beta-ol | 34% | B/B | -46.9 | -59.5 | -58.8 | -46.8 | -35.4 | **-43.0** | -39.3 | -13.9 |
| 270303608 | Q08IT1 | DADIS\_PANGI | 4.2.1.125 | dammarenediol II | 37% | B/B | -54.8 | **-58.6** | -45.6 | -50.5 | -30.3 | -23.9 | -20.4 | -8.8 |
| 300807978 | E2IUA8 | FRIES\_KALDA | 5.4.99.50 | friedelin | 40% | B/B | -36.3 | **-54.5** | n.p. | -36.7 | -34.4 | -33.7 | n.p. | -39.5 |
| 403377906 | B9X0J1 | STBOS\_STERE | 5.4.99.51 | baccharis oxide | 37% | B/B | -41.1 | **-47.6** | -20.0 | -37.2 | -36.2 | -29.7 | -20.8 | 0.9 |
| 350538549 | E7DN63 | BAMS\_SOLLC | 5.4.99.39 | beta-amyrin | 40% | B/B | -40.7 | **-47.0** | -30.1 | -38.0 | -37.5 | -36.6 | -60.0 | -31.9 |
| 300591997 | Q9LRH7 | ABAMS\_PEA | 5.4.99.39; 5.4.99.40 | beta-amyrin; alpha-amyrin | 38% | B/B | -44.2 | **-54.5** | -46.5 | -39.9 | -32.3 | -47.4 | -38.6 | -42.4 |
| 300807976 | E2IUA7 | GLUTS\_KALDA | 5.4.99.49 | glutinol | 39% | B/B | -46.0 | **-54.2** | -50.9 | -47.0 | -33.8 | -37.2 | -49.9 | -46.6 |
| 300591999 | Q9LRH8 | BAMS\_PEA | 5.4.99.39 | beta-amyrin | 40% | B/B | -42.7 | **-49.8** | -45.7 | -29.0 | -11.1 | -29.0 | -42.5 | -26.3 |
| 211926830 | B6EXY6 | LUP4\_ARATH | 5.4.99.39 | beta-amyrin | 39% | B/B | -38.5 | **-66.4** | -55.4 | -29.1 | -39.5 | -56.0 | -23.7 | -36.7 |
| 353558864 | A8C980 | GERS\_RHISY | 5.4.99.34 | germanicol | 41% | B/B | -42.1 | **-52.8** | n.p. | -50.2 | -49.2 | -44.5 | n.p. | -48.4 |
| 15233798 | O23390 | BARS1\_ARATH | 5.4.99.57 | baruol | 33% | B/B | -35.0 | -42.7 | -43.0 | -27.7 | -14.9 | **-36.3** | -32.9 | -24.1 |
| 15218390 | Q9SYN1 | PEN6\_ARATH | 5.4.99.52; 5.4.99.54 | alpha-seco-amyrin; beta-seco-amyrin | 35% | B/A | **-55.8** | -53.5 | -54.5 | -46.4 | -37.7 | -34.2 | -21.9 | -29.4 |
| 300592003 | Q9MB42 | BAMS\_GLYGL | 5.4.99.39 | beta-amyrin | 40% | B/C | -39.9 | n.p. | **-51.7** | -43.2 | -41.8 | n.p. | -48.0 | -35.0 |
| 300591979 | Q8W3Z2 | LUPS\_BETPL | 5.4.99.41 | lupeol | 39% | B/C | -59.3 | -47.0 | **-61.1** | -43.6 | -17.1 | -31.9 | -14.5 | -18.1 |
| 300807974 | E2IUA6 | TARS\_KALDA | 5.4.99.35 | taraxerol | 41% | B/C | -2.8 | -45.6 | **-58.7** | -39.3 | -20.2 | -17.2 | -23.3 | -24.3 |
| 30699380 | Q9C5M3 | LUP1\_ARATH | 4.2.1.128; 5.4.99.41 | lupan-3beta,20-diol; lupeol | 39% | B/C | -49.7 | -56.3 | **-62.9** | -39.0 | -43.6 | -45.5 | -41.4 | -28.5 |
| 257623103 | Q764T8 | LUPS\_GLYGL | 5.4.99.41 | lupeol | 39% | B/C | -43.0 | -48.5 | **-56.2** | -34.1 | -31.7 | -26.8 | -35.1 | -22.8 |
| 300591987 | Q948R6 | IMFS\_LUFCY | 5.4.99.36 | isomultiflorenol | 38% | B/C | -42.3 | n.p. | **-47.6** | -26.7 | -20.6 | n.p. | -12.2 | -33.6 |
| 353558692 | A8CDT3 | LUPS\_BRUGY | 5.4.99.41 | lupeol | 37% | B/A | **-47.2** | n.p. | -46.4 | -40.3 | -36.0 | n.p. | -38.8 | -39.6 |
| 300591977 | Q8W3Z1 | BAMS\_BETPL | 5.4.99.39 | beta-amyrin | 40% | B/C | -34.0 | n.p. | **-53.0** | -23.4 | -38.6 | n.p. | -39.9 | -41.8 |
| 260037884 | Q1G1A4 | LAS1\_ARATH | 5.4.99.7 | lanosterol | 40% | C/B | -59.6 | -59.5 | -31.3 | -45.7 | -32.4 | **-33.7** | -28.0 | -3.9 |
| 452446 | P38605 | CAS1\_ARATH | 5.4.99.8 | cycloartenol | 44% | C/A | **-85.4** | -78.6 | -81.5 | -71.7 | -44.4 | -42.7 | -49.0 | -56.5 |

Table S1e. Glide XP scores for the key intermediates of the crystal structures. The poses are the same as those in Table S3a.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PDB | A-I1 | B-I1 | C-I1 | D-I1 | A-I2 | B-I2 | C-I2 | D-I2 |
| 1SQC | **-9.509** | n.p. | -5.236 | n.p. | -4.612 | n.p. | -3.077 | n.p. |
| 1W6K | -9.079 | -9.134 | **-10.382** | -7.125 | -4.574 | -8.284 | -7.431 | -5.965 |