Table S1. Data for homologous positions in other subunits.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Struct | α1 | ϕ | kcal/mol | Ag | Ref | β1 | ϕ | kcal/mol | Ag | Ref | δ | ϕ | kcal/mol | Ag | Ref | ε | ϕ | kcal/mol | Ag | Ref |
| PP & Col | **\*E45** | **0.77** | **3.09** | both | 16 | E45 | 0.25 | 0.47 | ACh | 13 | E47 | 0.15 | 0.42 | ACh | 13 | **E45** | **0.5** | **0.98** | Cho | 3 |
| PP | **V46** | **0.92** | **1.82** | ACh | 13 | K46 | 0.29 | 0.43 | Cho |  | V48 | 2.59 | 0.03 | ACh | 13 | K46 | -0.8 | 0.15 | ACh | 13 |
| loop D | R55 | 0.66 | 0.24 | Cho | 5 | Y55 | 0.56 | 0.24 | Cho |  | **W57** | **0.94** | **1.67** | ACh | 2 | **W55** | **0.97** | **1.67** | ACh | 2 |
| loop A | **Y93** | **0.88** | **2.31** | ACh | 19 | L93 | 0.52 | 0.47 | Cho |  |  |  |  |  |  |  |  |  |  |  |
| Col | **A96** | **0.79** | **4.21** | both | 4 | N96 | 0.07 | 0.52 | Cho |  | **N98** | **0.44** | **0.98** | Cho | 6 |  |  |  |  |  |
|  | **\*D97** | **0.93** | **1.75** | Cho | 6 | D97 | 0.31 | 0.42 | Cho | 6 | D99 | 0.32 | 0.55 | Cho | 6 | D97 | 0.31 | 0.11 | Cho | 6 |
|  | **\*G98** | **nd** | **1.44** | Cho | 6 | G98 | 0.4 | 0.68 | Cho | 6 |  |  |  |  |  |  |  |  |  |  |
| Col | **Y127** | **0.77** | **3.71** | both | 18 | S127 | -0.73 | 0.65 | Cho | 18 | S129 | -0.69 | 0.62 | ACh | 18 | T127 | -0.08 | 0.64 | both | 18 |
|  | **\*V132** | **0.75** | **2.34** | ACh | 9 | **V132** | **-0.12** | **0.77** | Cho |  |  |  |  |  |  |  |  |  |  |  |
|  | **\*F135** | **0.75** | **0.85** | both | 9 | F135 | -0.69 | 0.17 | ACh | 12 | **F137** | **0.53** | **1.47** | ACh | 12 | **F135** | **0.57** | **1.31** | ACh | 12 |
|  | \*F137 | 0.53 | 0.45 | Cho | 5 | F137 | -1.34 | 0.07 | ACh | 12 | F139 | 0.39 | 0.52 | ACh | 12 | F137 | 0.96 | 0.17 | ACh | 12 |
| loop B & Col | **W149** | **0.94** | **2.74** | ACh | 15 | Y149 | 0.73 | 0.48 | Cho |  |  |  |  |  |  |  |  |  |  |  |
| loop9 | S173 | 0.14 | 0.47 | both | 10 | N182 | 0.15 | 0.18 | Cho |  |  |  |  |  |  | **N182** | **0.78** | **2.09** | Cho | 10 |
| loop9 | **\*G174** | **0.82** | **1.08** | both | 10 | G183 | 0.69 | 0.55 | Cho |  |  |  |  |  |  | **G183** | **0.97** | **1.30** | Cho | 10 |
| loop9 | **E175** | **0.52** | **1.04** | ACh | 10 | Q184 | -0.37 | 0.40 | Cho |  |  |  |  |  |  | **E184** | **0.60** | **3.27** | Cho | 10 |
| Pre-M1 | M207 | 0.05 | 0.24 | Cho | 16 | I218 | 0.69 | 0.25 | Cho |  |  |  |  |  |  |  |  |  |  |  |
| Pre-M1 | Q208 | 0.64 | 0.51 | Cho | 16 | **R219** | **0.27** | **0.76** | Cho |  |  |  |  |  |  | R217 | 0.59 | 0.34 | Cho | 3 |
| Pre-M1 & PP | **\*R209** | **0.72** | **1.24** | both | 16 | R220 | 6.88 | 0.02 | Cho |  | **R223** | **0.54** | **1.99** | ACh | 3 | **R218** | **0.73** | **3.06** | ACh | 3 |
| Pre-M1 | **L210** | **0.35** | **1.17** | Cho | 16 | K221 | 0.52 | 0.54 | Cho |  | K224 | 0.82 | 0.31 | ACh | 12 | **K219** | **0.63** | **1.14** | Cho | 3 |
| TM2 | **S246** | **0.63** | **1.44** | Cho | 17 | S257 | -0.05 | 0.67 | Cho |  |  |  |  |  |  | **S256** | **0.67** | **1.36** | ACh | 11 |
| TM2 | **V249** | **0.50** | **1.24** | Cho | 17 | **A260** | **0.03** | **0.80** | Cho |  | **V263** | **-0.08** | **0.82** | Cho | 7 | **V259** | **0.58** | **1.71** | both | 11 |
| TM2 | **\*L251** | **0.26** | **1.95** | both | 17 | **L262** | **0.37** | **2.08** | Cho | 14 | **L265** | **0.03** | **1.85** | Cho | 7 | **L261** | **0.37** | **4.20** | Cho | 11 |
| TM2 | **T254** | **0.35** | **1.93** | Cho | 17 | **T265** | **0.42** | **0.93** | Cho |  | **S268** | **0.28** | **4.08** | Cho | 7 | **T264** | **0.26** | **5.29** | Cho | 11 |
| TM2 & Col | **\*V255** | **0.52** | **2.75** | both | 17 | **V266** | **0.48** | **2.01** | Cho |  | **V269** | **0.24** | **4.13** | Cho | 7 | **V265** | **0.34** | **5.69** | both | 11 |
| TM2 | **V259** | **0.63** | **1.40** | Cho | 14 | **L270** | **0.53** | **2.80** | Cho |  | **L273** | **0.37** | **3.16** | Cho | 7 | **L269** | **0.52** | **3.06** | Cho | 11 |
| TM2-TM3 | **I264** | **0.78** | **2.31** | both | 1 | **V275** | **0.24** | **1.19** | Cho |  |  |  |  |  |  | **I274** | **0.62** | **1.20** | both | 11 |
| TM2-TM3 & Col | **\*P265** | **0.90** | **2.72** | both | 1 | **P276** | **0.24** | **1.26** | Cho |  |  |  |  |  |  | **P275** | **0.51** | **1.60** | Cho | 11 |
| TM2-TM3 & Col | **\*S268** | **0.97** | **2.50** | both | 1 | **S279** | **0.43** | **1.85** | Cho | 8 |  |  |  |  |  | **S278** | **-0.04** | **0.85** | both | 11 |
| TM2-TM3 & PP | **S269** | **0.69** | **1.40** | Cho | 8 | L280 | -0.3 | 0.50 | Cho |  | M283 | -0.15 | 0.07 | ACh | 13 | L279 | -0.07 | 0.30 | ACh | 13 |
| TM2-TM3 | **A270** | **0.65** | **1.48** | both | 9 | A281 | 0.34 | 0.45 | Cho |  |  |  |  |  |  | S280 | 0.31 | 0.59 | ACh | 11 |
| TM2-TM3 | V271 | -0.76 | 0.20 | both | 9 | V282 | 0.31 | 0.67 | Cho |  |  |  |  |  |  |  |  |  |  |  |
| TM2-TM3 & Col & PP | **\*P272** | **0.62** | **2.83** | both | 9 | P283 | 0.16 | 0.62 | Cho |  | P286 | -0.43 | 0.30 | ACh | 13 | P282 | -5.98 | 0.03 | ACh | 13 |
| TM2-TM3 | L273 | 0.54 | 0.44 | ACh | 9 | **I284** | **0.52** | **0.78** | Cho |  | L287 | 0.01 | 0.38 | ACh | 12 | L283 | 0.67 | 0.18 | ACh | 12 |
| TM2-TM3 | **I274** | **0.62** | **2.24** | ACh | 9 | **I285** | **0.24** | **1.44** | Cho |  |  |  |  |  |  |  |  |  |  |  |
| TM2-TM3 | **G275** | **0.65** | **1.32** | ACh | 9 | **I286** | **0.48** | **1.03** | Cho |  |  |  |  |  |  |  |  |  |  |  |

**Table S1. Data for homologous positions in all subunits.**

The first column shows structural elements in the α subunit (see Text). The following columns show data for each subunit. The residue and position (an asterisk indicates the homologous residues are identical in all subunits), the value of ϕ, the range energy and the source for the data (no source listed indicates data from the present study). When the range energy at a position is greater than 0.7 kcal/mol values are in **bold**. In some cases values for range energy and φ were calculated from data provided in the reference. The column headed "Ag" gives the agonist used in the study: ACh: acetylcholine, Cho: choline, both: both agonists used on different constructs. Ref provides the reference in the list below. If no reference is given then the data are from the present study.

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