# Understanding G protein selectivity of muscarinic acetylcholine receptors using computational methods

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## Supplementary Information

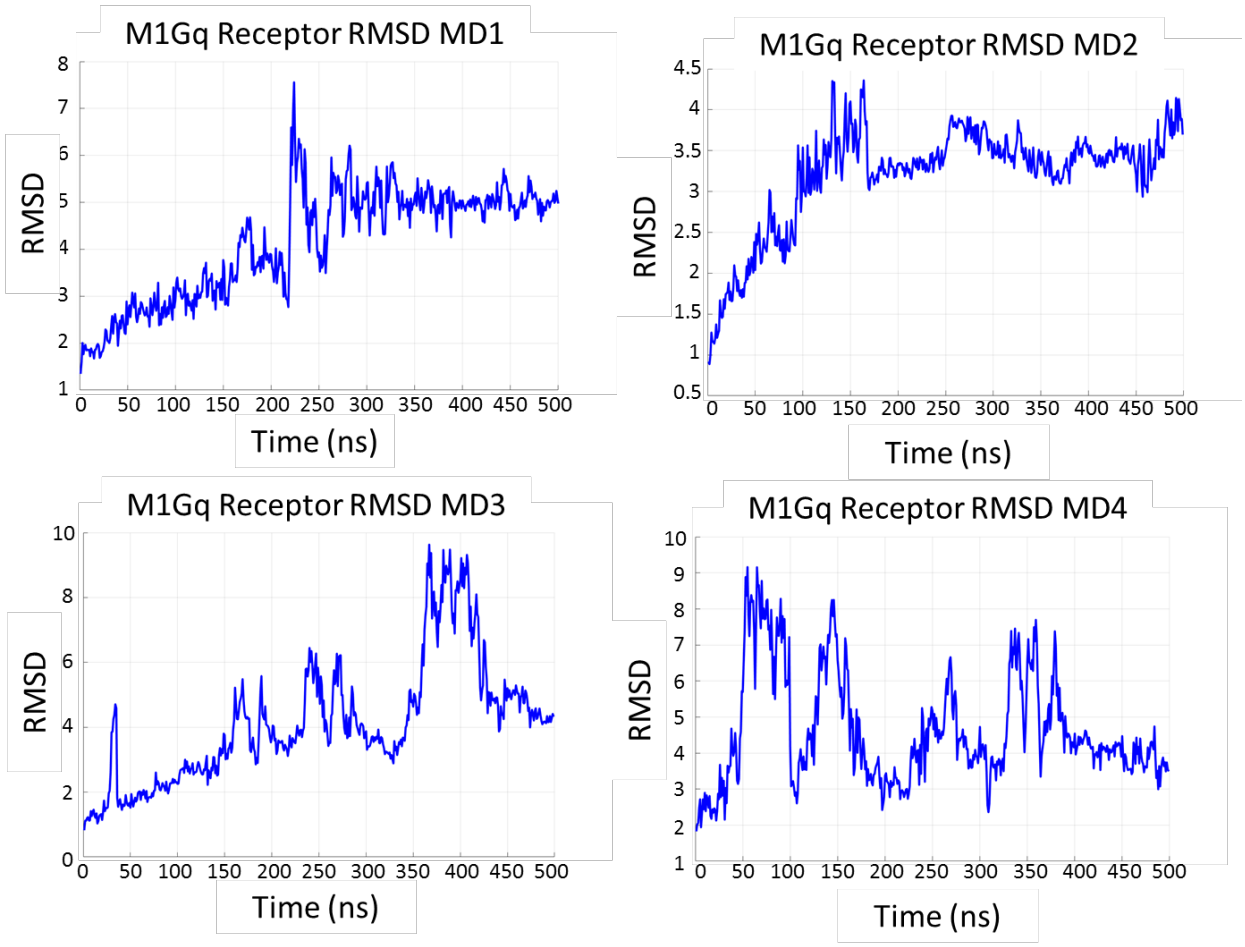


**Figure S1:** RMSD of the M1 Receptor in M1:Gαq complex for 4x500ns MD simulations.

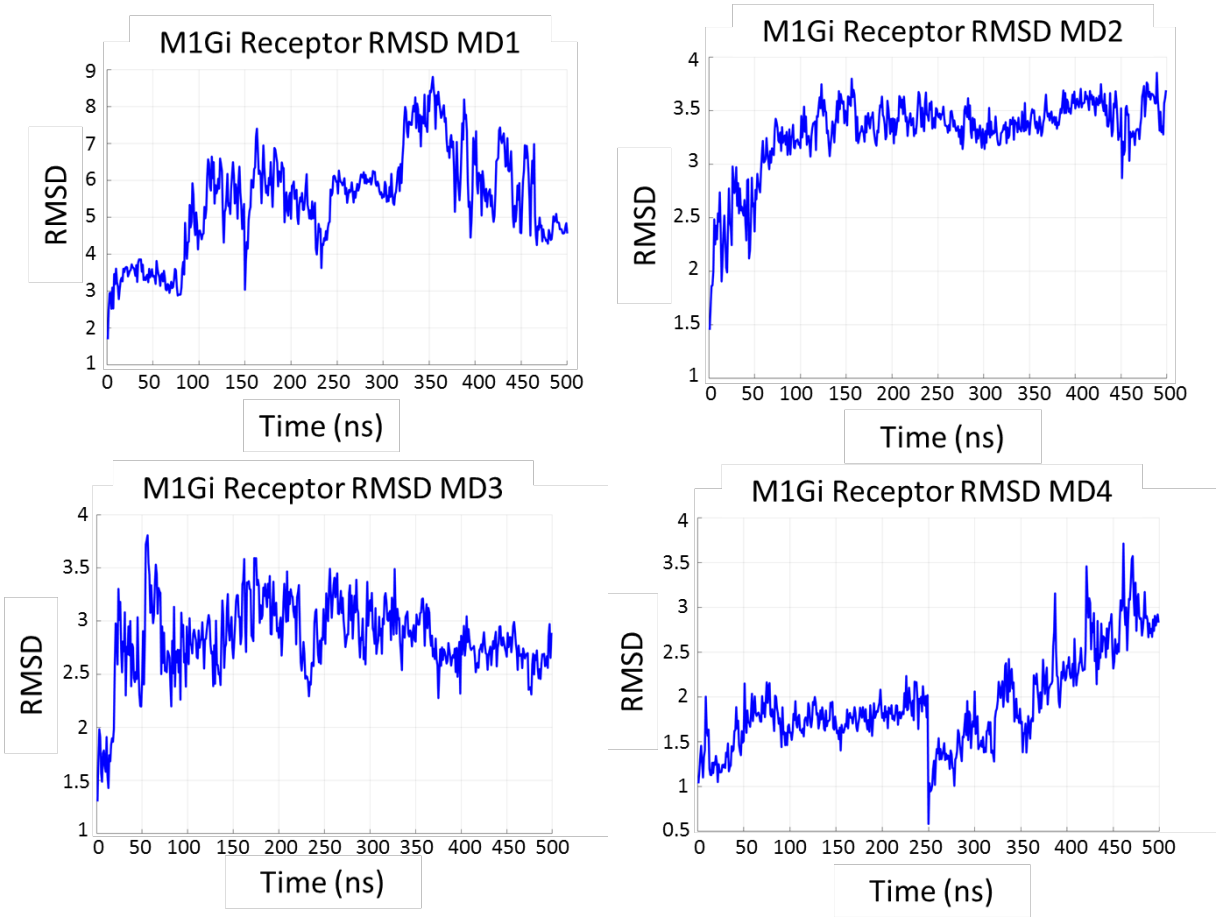


**Figure S2:** RMSD of the M1 Receptor in M1:Gαi complex for 4x500ns MD simulations.

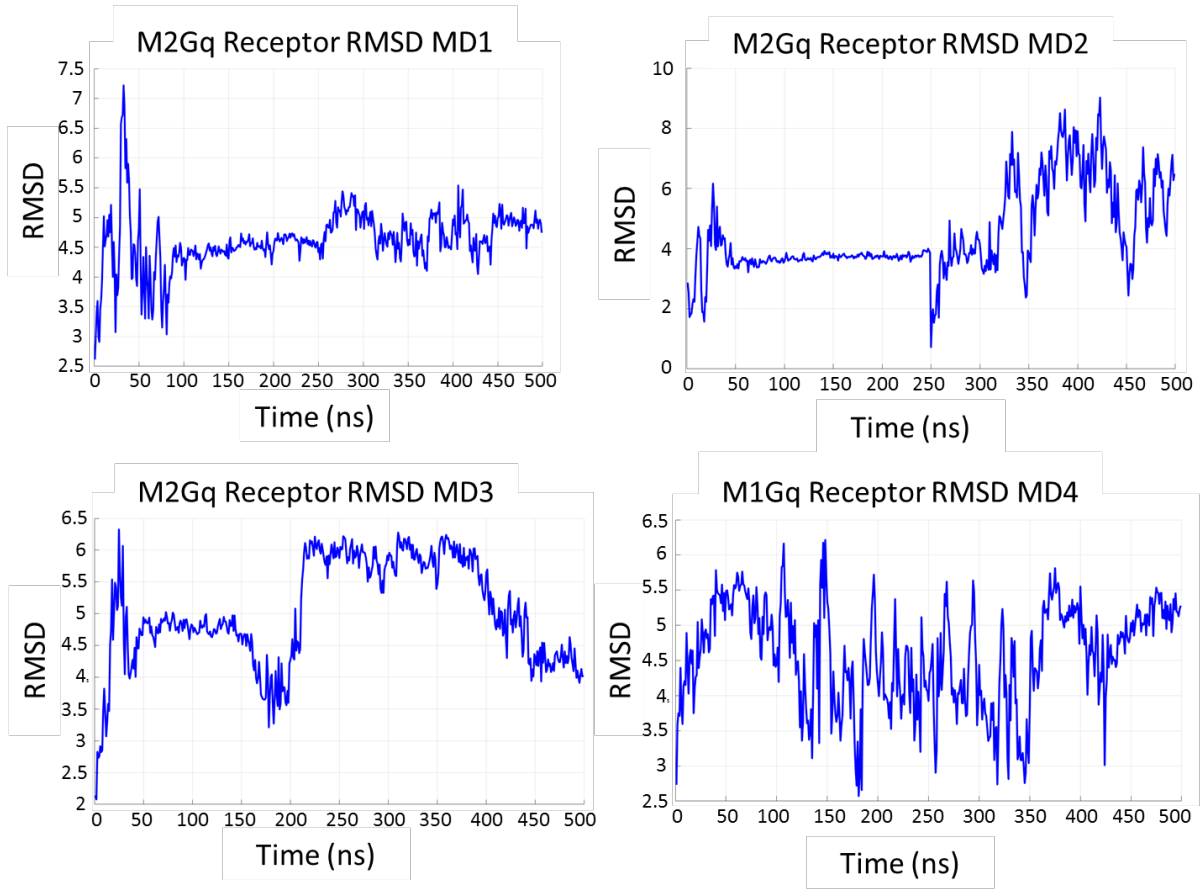


**Figure S3:** RMSD of the M2 Receptor in M2:Gαq complex for 4x500ns MD simulations.

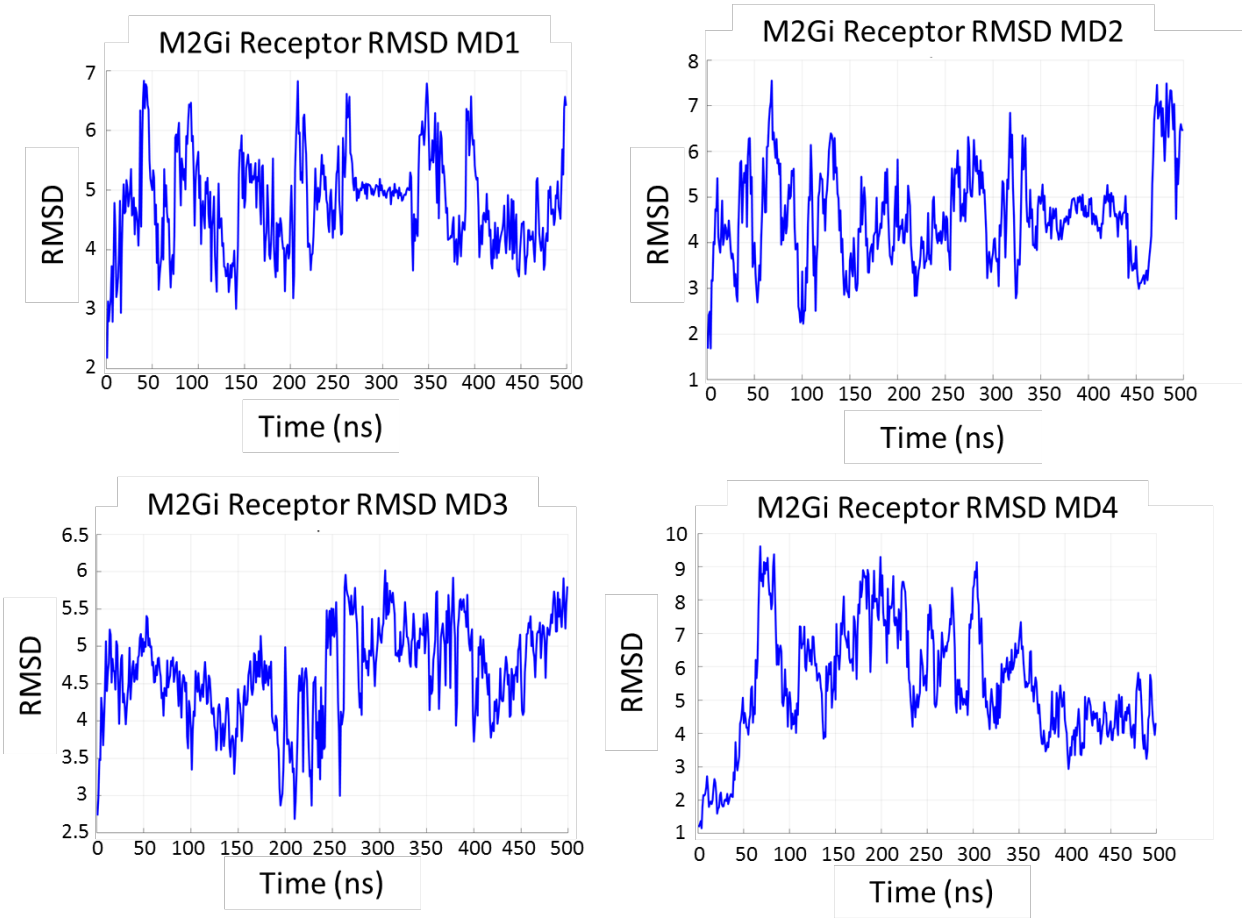


**Figure S4:** RMSD of the M2 Receptor in M2:Gαi complex for 4x500ns MD simulations.

| Domains | M1R | M2R |
|---|---|---|
| TM1 | 23-52 | 21-50 |
| TM2 | 59-87 | 57-85 |
| TM3 | 95-128 | 93-126 |
| TM4 | 139-163 | 137-161 |
| TM5 | 184-217 | 182-215 |
| TM6 | 358-390 | 380-412 |
| TM7 | 395-421 | 417-443 |
| 1.50 Residue | N43 | N41 |
| 2.50 Residue | D71 | D69 |
| 3.50 Residue | R123 | R121 |
| 4.50 Residue | W150 | W148 |
| 5.50 Residue | P200 | P198 |
| 6.50 Residue | P380 | P402 |
| 7.50 Residue | P415 | P437 |

**Table S0:** The residue numbers for the TM domains for the two receptors are shown along with the most conserved residue in each TM domain that defines the Ballesteros-Weinstein numbering scheme, useful for comparing different receptors [1].

Hydrogen bonds | Residues in M1(AALS)/M2(VTIL) motif

Key M2 interaction | Mentioned in M1/M2 cryo-EM paper

Key M1 interaction | Common across all systems

| M1:Gq | | | M2:Gq | | | M1:Gi | | | M2:Gi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BW | GN | Pop | BW | GN | Pop | BW | GN | Pop | BW | GN | Pop |
| ARG123$^{3.50}$ | TYR356$^{G.H5.23}$ | 99% | ARG121$^{3.50}$ | TYR356$^{G.H5.23}$ | 94% | ARG123$^{3.50}$ | CYS352$^{G.H5.23}$ | 73% | ARG121$^{3.50}$ | CYS352$^{G.H5.23}$ | 87% |
| SER126$^{3.53}$ | ASN352$^{G.H5.19}$ | 77% | CYS124$^{3.53}$ | ASN352$^{G.H5.19}$ | 84% | SER126$^{3.53}$ | ASN348$^{G.H5.19}$ | 89% | CYS124$^{3.53}$ | ASN348$^{G.H5.19}$ | 96% |
| LYS362$^{6.32}$ | VAL359$^{G.H5.26}$ | 92% | LYS384$^{6.32}$ | VAL359$^{G.H5.26}$ | 81% | LYS362$^{6.32}$ | PHE355$^{G.H5.26}$ | 91% | LYS384$^{6.32}$ | PHE355$^{G.H5.26}$ | 68% |
| ALA363$^{6.33}$ | LEU358$^{G.H5.25}$ | 89% | VAL385$^{6.33}$ | LEU358$^{G.H5.25}$ | 86% | ALA363$^{6.33}$ | LEU354$^{G.H5.25}$ | 76% | VAL385$^{6.33}$ | LEU354$^{G.H5.25}$ | 71% |
| VAL127$^{3.54}$ | LEU349$^{G.H5.16}$ | 83% | VAL125$^{3.54}$ | LEU349$^{G.H5.16}$ | 76% | VAL127$^{3.54}$ | ILE345$^{G.H5.16}$ | 76% | VAL125$^{3.54}$ | ILE345$^{G.H5.16}$ | 47% |
| THR366$^{6.36}$ | LEU358$^{G.H5.25}$ | 60% | THR388$^{6.36}$ | LEU358$^{G.H5.25}$ | 79% | THR366$^{6.36}$ | LEU354$^{G.H5.25}$ | 53% | THR388$^{6.36}$ | LEU354$^{G.H5.25}$ | 70% |
| CYS421$^{7.56}$ | ASN357$^{G.H5.24}$ | 78% | CYS443$^{7.56}$ | ASN357$^{G.H5.24}$ | 77% | CYS421$^{7.56}$ | GLY353$^{G.H5.24}$ | 38% | CYS443$^{7.56}$ | GLY353$^{G.H5.24}$ | 48% |
| SER126$^{3.53}$ | TYR356$^{G.H5.23}$ | 99% | CYS124$^{3.53}$ | TYR356$^{G.H5.23}$ | 98% | | | 0% | CYS124$^{3.53}$ | CYS352$^{G.H5.23}$ | 42% |
| PRO130$^{IC2}$ | ASN348$^{G.H5.15}$ | 32% | PRO128$^{IC2}$ | ASN348$^{G.H5.15}$ | 85% | PRO130$^{IC2}$ | ILE344$^{G.H5.15}$ | 68% | PRO128$^{IC2}$ | ILE344$^{G.H5.15}$ | 46% |
| LYS362$^{6.32}$ | LEU358$^{G.H5.25}$ | 61% | LYS384$^{6.32}$ | LEU358$^{G.H5.25}$ | 81% | LYS362$^{6.32}$ | LEU354$^{G.H5.25}$ | 44% | LYS384$^{6.32}$ | LEU354$^{G.H5.25}$ | 32% |
| LEU131$^{IC2}$ | VAL199$^{G.S3.1}$ | 35% | LEU129$^{IC2}$ | VAL199$^{G.S3.1}$ | 58% | LEU131$^{IC2}$ | LEU195$^{G.S3.1}$ | 76% | LEU129$^{IC2}$ | LEU195$^{G.S3.1}$ | 39% |
| PRO130$^{IC2}$ | ASN352$^{G.H5.19}$ | 29% | PRO128$^{IC2}$ | ASN352$^{G.H5.19}$ | 53% | PRO130$^{IC2}$ | ASN348$^{G.H5.19}$ | 56% | PRO128$^{IC2}$ | ASN348$^{G.H5.19}$ | 64% |
| LEU131$^{IC2}$ | LYS345$^{G.H5.12}$ | 54% | LEU129$^{IC2}$ | LYS345$^{G.H5.12}$ | 42% | LEU131$^{IC2}$ | THR341$^{G.H5.12}$ | 77% | LEU129$^{IC2}$ | THR341$^{G.H5.12}$ | 27% |
| THR215$^{5.65}$ | LEU353$^{G.H5.20}$ | 63% | SER213$^{5.65}$ | LEU353$^{G.H5.20}$ | 50% | THR215$^{5.65}$ | LEU349$^{G.H5.20}$ | 74% | | | 0% |
| THR366$^{6.36}$ | ASN357$^{G.H5.24}$ | 71% | THR388$^{6.36}$ | ASN357$^{G.H5.24}$ | 52% | THR366$^{6.36}$ | GLY353$^{G.H5.24}$ | 60% | | | 0% |
| LEU367$^{6.37}$ | LEU358$^{G.H5.25}$ | 27% | ILE389$^{6.37}$ | LEU358$^{G.H5.25}$ | 49% | LEU367$^{6.37}$ | LEU354$^{G.H5.25}$ | 52% | ILE389$^{6.37}$ | LEU354$^{G.H5.25}$ | 51% |
| LEU131$^{IC2}$ | PHE341$^{G.H5.8}$ | 36% | LEU129$^{IC2}$ | PHE341$^{G.H5.8}$ | 52% | LEU131$^{IC2}$ | PHE337$^{G.H5.8}$ | 48% | LEU129$^{IC2}$ | PHE337$^{G.H5.8}$ | 25% |
| LEU131$^{IC2}$ | ASN348$^{G.H5.15}$ | 50% | | | 0% | LEU131$^{IC2}$ | ILE344$^{G.H5.15}$ | 57% | LEU129$^{IC2}$ | ILE344$^{G.H5.15}$ | 54% |
| ASN422$^{CTER}$ | GLU355$^{G.H5.22}$ | 81% | | | 0% | ASN422$^{CTER}$ | ASP351$^{G.H5.22}$ | 72% | | | 0% |
| PRO130$^{IC2}$ | LEU349$^{G.H5.16}$ | 25% | | | 0% | PRO130$^{IC2}$ | ILE345$^{G.H5.16}$ | 74% | PRO128$^{IC2}$ | ILE345$^{G.H5.16}$ | 47% |
| ALA135$^{IC2}$ | ARG37$^{G.Hns1.2}$ | 20% | | | 0% | ALA135$^{IC2}$ | ALA31$^{G.Hns1.2}$ | 56% | VAL133$^{IC2}$ | ALA31$^{G.Hns1.2}$ | 50% |
| ARG134$^{IC2}$ | ASN352$^{G.H5.19}$ | 64% | | | 0% | ARG134$^{IC2}$ | ASN348$^{G.H5.19}$ | 24% | PRO132$^{IC2}$ | ASN348$^{G.H5.19}$ | 34% |
| VAL127$^{3.54}$ | LEU353$^{G.H5.20}$ | 32% | | | 0% | VAL127$^{3.54}$ | LEU349$^{G.H5.20}$ | 35% | VAL125$^{3.54}$ | LEU349$^{G.H5.20}$ | 53% |
| THR218$^{5.68}$ | LEU349$^{G.H5.16}$ | 37% | | | 0% | THR218$^{5.68}$ | ILE345$^{G.H5.16}$ | 78% | | | 0% |
| THR218$^{5.68}$ | ASP346$^{G.H5.13}$ | 40% | | | 0% | THR218$^{5.68}$ | ASP342$^{G.H5.13}$ | 73% | | | 0% |
| ALA135$^{IC2}$ | ARG38$^{G.Hns1.3}$ | 55% | | | 0% | ALA135$^{IC2}$ | ARG32$^{G.Hns1.3}$ | 54% | | | 0% |
| ILE211$^{5.61}$ | LEU358$^{G.H5.25}$ | 38% | | | 0% | ILE211$^{5.61}$ | LEU354$^{G.H5.25}$ | 59% | | | 0% |
| | | 0% | ARG381$^{6.29}$ | GLN350$^{G.H5.17}$ | 86% | | | 0% | | | 0% |
| ARG134$^{IC2}$ | ILE348$^{G.H5.15}$ | 29% | | | 0% | ARG134$^{IC2}$ | ILE344$^{G.H5.15}$ | 53% | | | 0% |
| | | 0% | VAL385$^{6.33}$ | VAL359$^{G.H5.26}$ | 55% | | | 0% | VAL385$^{6.33}$ | PHE355$^{G.H5.26}$ | 26% |
| | | 0% | ASN444$^{CTER}$ | ASN357$^{G.H5.24}$ | 76% | | | 0% | | | 0% |
| | | 0% | ASN444$^{CTER}$ | GLU355$^{G.H5.22}$ | 75% | | | 0% | | | 0% |
| | | 0% | | | 0% | | | 0% | LYS214$^{5.66}$ | ASP342$^{G.H5.13}$ | 74% |
| | | 0% | | | 0% | | | 0% | LYS134$^{IC2}$ | GLU28$^{G.HN.52}$ | 73% |
| | | 0% | ARG381$^{6.29}$ | ASP346$^{G.H5.13}$ | 73% | | | 0% | | | 0% |
| | | 0% | | | 0% | ARG134$^{IC2}$ | ALA31$^{G.Hns1.2}$ | 57% | | | 0% |
| ARG137$^{IC2}$ | ARG37$^{G.Hns1.2}$ | 35% | | | | | | | | | |
| THR367$^{6.37}$ | LEU358$^{G.H5.25}$ | 27% | | | | | | | | | |

**Table S1:** Receptor—G-protein noncovalent interactions in the four receptor—G-protein complexes. Residue names and numbers are followed by their Ballesteros-Weinstein numbers for GPCRs [1], common Gα numbering system for Gα protein residues [2] and % of the 2 μs simulation time that those interactions are observed.

| Conserved | M1 Unique | From Literature | M2 Unique |
|---|---|---|---|

| M1 Systems | | | | | | M1:Gq | M1:Gi | M2 systems | | | | | | M2:Gq | M2:Gi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ASP | 71 | 2.50 | SER | 411 | 7.46 | 84% | 94% | ASP | 69 | 2.50 | SER | 433 | 7.46 | 91% | 94% |
| GLN | 110 | 3.37 | TRP | 157 | 4.57 | 93% | 84% | ASN | 108 | 3.37 | TRP | 155 | 4.57 | 89% | 72% |
| THR | 39 | 1.46 | THR | 412 | 7.47 | 87% | 88% | THR | 37 | 1.46 | THR | 434 | 7.47 | 78% | 75% |
| GLN | 110 | 3.37 | SER | 153 | 4.53 | 80% | 77% | ASN | 108 | 3.37 | SER | 151 | 4.53 | 82% | 88% |
| SER | 78 | 2.57 | ASP | 105 | 3.32 | 79% | 78% | SER | 76 | 2.57 | ASP | 103 | 3.32 | 82% | 86% |
| ASN | 43 | 1.50 | ALA | 68 | 2.47 | 73% | 81% | ASN | 41 | 1.50 | ALA | 66 | 2.47 | 74% | 92% |
| TYR | 106 | 3.33 | TRP | 157 | 4.57 | 84% | 76% | TYR | 104 | 3.33 | TRP | 155 | 4.57 | 82% | 76% |
| SER | 36 | 1.43 | ASN | 80 | 2.59 | 68% | 71% | SER | 34 | 1.43 | ASN | 78 | 2.59 | 80% | 97% |
| PHE | 125 | 3.52 | TYR | 133 | ICL2 | 95% | 55% | PHE | 123 | 3.52 | TYR | 131 | ICL2 | 85% | 81% |
| TYR | 85 | 2.64 | CYS | 177 | ECL2 | 64% | 74% | TYR | 83 | 2.64 | CYS | 176 | ECL2 | 92% | 85% |
| PHE | 121 | 3.48 | LEU | 207 | 5.57 | 71% | 79% | PHE | 119 | 3.48 | LEU | 205 | 5.57 | 80% | 79% |
| ALA | 111 | 3.38 | SER | 153 | 4.53 | 77% | 77% | ALA | 109 | 3.38 | SER | 151 | 4.53 | 78% | 77% |
| SER | 66 | 2.45 | ASN | 115 | 3.42 | 74% | 82% | SER | 64 | 2.45 | ASN | 113 | 3.42 | 70% | 82% |
| ASN | 43 | 1.50 | ASP | 71 | 2.50 | 79% | 74% | ASN | 41 | 1.50 | ASP | 69 | 2.50 | 82% | 71% |
| CYS | 98 | 3.25 | CYS | 178 | ECL2 | 86% | 79% | CYS | 96 | 3.25 | CYS | 176 | ECL2 | 70% | 68% |
| ALA | 111 | 3.38 | TRP | 150 | 4.50 | 64% | 73% | ALA | 109 | 3.38 | TRP | 148 | 4.50 | 84% | 81% |
| ASP | 99 | 3.26 | ARG | 171 | ECL2 | 83% | 58% | ASP | 97 | 3.26 | ARG | 169 | ECL2 | 85% | 72% |
| ASP | 122 | 3.49 | TYR | 133 | ICL2 | 66% | 89% | ASP | 120 | 3.49 | TYR | 131 | ICL2 | 78% | 66% |
| **TYR** | **208** | **5.58** | **LEU** | **367** | **6.37** | **71%** | **69%** | **TYR** | **206** | **5.58** | **ILE** | **389** | **6.37** | **76%** | **81%** |
| VAL | 107 | 3.34 | SER | 153 | 4.53 | 64% | 74% | VAL | 105 | 3.34 | SER | 151 | 4.53 | 76% | 82% |
| LEU | 81 | 2.60 | TRP | 101 | 3.28 | 65% | 80% | LEU | 79 | 2.60 | TRP | 99 | 3.28 | 74% | 78% |
| TYR | 106 | 3.33 | TYR | 381 | 6.51 | 57% | 86% | TYR | 104 | 3.33 | TYR | 403 | 6.51 | 68% | 82% |
| TRP | 377 | 6.47 | ASN | 410 | 7.45 | 80% | 61% | THR | 399 | 6.47 | ASN | 432 | 7.45 | 66% | 83% |
| TYR | 106 | 3.33 | ILE | 180 | ECL2 | 77% | 81% | TYR | 104 | 3.33 | ILE | 178 | ECL2 | 60% | 70% |
| SER | 78 | 2.57 | TRP | 101 | 3.28 | 71% | 52% | SER | 76 | 2.57 | TRP | 99 | 3.28 | 83% | 77% |
| SER | 36 | 1.43 | GLY | 75 | 2.54 | 56% | 78% | SER | 34 | 1.43 | GLY | 73 | 2.54 | 69% | 79% |
| PHE | 197 | 5.47 | TRP | 379 | 6.48 | 80% | 65% | PHE | 195 | 5.47 | TRP | 400 | 6.48 | 70% | 64% |
| LEU | 183 | ECL2 | THR | 189 | 5.39 | 56% | 74% | PHE | 181 | ECL2 | THR | 187 | 5.39 | 71% | 79% |
| TYR | 124 | 3.51 | LEU | 207 | 5.57 | 79% | 67% | TYR | 122 | 3.51 | LEU | 205 | 5.57 | 61% | 71% |
| TYR | 124 | 3.51 | ARG | 210 | 5.60 | 71% | 65% | TYR | 122 | 3.51 | HSD | 208 | 5.60 | 72% | 70% |
| ASN | 43 | 1.50 | PRO | 415 | 7.50 | 73% | 75% | ASN | 41 | 1.50 | PRO | 437 | 7.50 | 67% | 60% |
| PHE | 63 | 2.42 | LEU | 118 | 3.45 | 67% | 61% | PHE | 61 | 2.42 | ILE | 116 | 3.45 | 71% | 75% |
| THR | 189 | 5.39 | THR | 389 | 6.59 | 63% | 74% | THR | 187 | 5.39 | THR | 411 | 6.59 | 72% | 61% |
| TRP | 91 | ECL1 | TRP | 101 | 3.28 | 56% | 56% | TRP | 89 | ECL1 | TRP | 99 | 3.28 | 80% | 75% |
| THR | 32 | 1.39 | THR | 83 | 2.62 | 74% | 72% | ALA | 30 | 1.39 | THR | 81 | 2.62 | 62% | 57% |
| LEU | 56 | ICL1 | ASN | 61 | 2.40 | 82% | 76% | LEU | 54 | ICL1 | ASN | 59 | 2.40 | 87% | 19% |
| PHE | 197 | 5.47 | ASN | 382 | 6.52 | 52% | 73% | PHE | 195 | 5.47 | ASN | 404 | 6.52 | 60% | 78% |
| SER | 126 | 3.53 | TYR | 133 | ICL2 | 68% | 55% | CYS | 124 | 3.53 | TYR | 131 | ICL2 | 66% | 63% |
| ASN | 60 | 2.39 | ASP | 122 | 3.49 | 73% | 65% | ASN | 58 | 2.39 | ASP | 120 | 3.49 | 70% | 43% |
| TYR | 124 | 3.51 | ILE | 211 | 5.61 | 64% | 69% | TYR | 122 | 3.51 | ILE | 209 | 5.61 | 67% | 51% |
| ALA | 160 | 4.60 | PHE | 182 | ECL2 | 73% | 65% | ALA | 158 | 4.60 | PHE | 180 | ECL2 | 58% | 54% |
| SER | 49 | 1.56 | THR | 428 | Cter | 72% | 76% | SER | 47 | 1.56 | THR | 450 | Cter | 61% | 40% |
| TYR | 82 | 2.61 | GLU | 401 | 7.36 | 60% | 54% | TYR | 80 | 2.61 | THR | 423 | 7.36 | 70% | 63% |
| TRP | 164 | ECL2 | GLN | 181 | ECL2 | 86% | 0% | TRP | 162 | ECL2 | GLN | 179 | ECL2 | 77% | 83% |
| PHE | 50 | 1.57 | LYS | 57 | ICI1 | 77% | 87% | ILE | 48 | 1.57 | GLN | 55 | ICL1 | 74% | 5% |
| PHE | 50 | 1.57 | LEU | 65 | 2.44 | 59% | 69% | ILE | 48 | 1.57 | PHE | 63 | 2.44 | 47% | 64% |
| SER | 120 | 3.47 | LEU | 207 | 5.57 | 54% | 66% | SER | 118 | 3.47 | LEU | 205 | 5.57 | 66% | 50% |
| ASN | 43 | 1.50 | LEU | 72 | 2.51 | 57% | 73% | ASN | 41 | 1.50 | LEU | 70 | 2.51 | 48% | 51% |
| PHE | 50 | 1.57 | LEU | 56 | ICL1 | 63% | 66% | ILE | 48 | 1.57 | LEU | 54 | ICL1 | 66% | 34% |
| TYR | 85 | 2.64 | GLN | 176 | ECL2 | 0% | 65% | TYR | 83 | 2.64 | GLU | 175 | ECL2 | 80% | 79% |
| TRP | 91 | ECL1 | CYS | 98 | 3.25 | 56% | 0% | TRP | 89 | ECL1 | CYS | 96 | 3.25 | 85% | 77% |
| TYR | 85 | 2.64 | HSD | 90 | ECL1 | 79% | 66% | TYR | 83 | 2.64 | TRP | 89 | ECL1 | 62% | 73% |
| TYR | 82 | 2.61 | TYR | 408 | 7.43 | 68% | 50% | TYR | 80 | 2.61 | TYR | 430 | 7.43 | 33% | 50% |
| MET | 384 | 6.54 | TRP | 400 | 7.35 | 57% | 59% | MET | 406 | 6.54 | TRP | 422 | 7.35 | 76% | 63% |

**Table S2** contd…

| Conserved in Cognate Complexes Only | M1 Unique | Conserved | M2 Unique |
|---|---|---|---|

| M1 Systems | | | | | | M1:Gq | M1:Gi | M2 systems | | | | | | M2:Gq | M2:Gi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TYR | 212 | 5.62 | ALA | 364 | 6.34 | 58% | 0% | SER | 210 | 5.62 | THR | 386 | 6.34 | 74% | 58% |
| PRO | 186 | 5.36 | THR | 390 | 6.59 | 60% | 69% | ALA | 184 | 5.36 | THR | 411 | 6.59 | 0% | 59% |
| ALA | 70 | 2.49 | ALA | 108 | 3.35 | 50% | 0% | ALA | 68 | 2.49 | VAL | 106 | 3.35 | 68% | 70% |
| GLY | 42 | 1.49 | PRO | 415 | 7.50 | 0% | 58% | GLY | 40 | 1.49 | PRO | 437 | 7.50 | 56% | 71% |
| SER | 49 | 1.56 | PHE | 425 | Cter | 73% | 0% | SER | 47 | 1.56 | PHE | 447 | Cter | 46% | 65% |
| TYR | 82 | 2.61 | TYR | 404 | 7.39 | 0% | 52% | TYR | 80 | 2.61 | TYR | 426 | 7.39 | 69% | 61% |
| LYS | 51 | 1.58 | LYS | 57 | ICL1 | 58% | 65% | LYS | 49 | 1.58 | GLN | 55 | ICL1 | 54% | 5% |
| | | | | | | | | **ASP** | **103** | **3.32** | **TYR** | **430** | **7.43** | **87%** | **88%** |
| SER | 49 | 1.56 | LEU | 56 | ICL1 | 50% | 57% | SER | 47 | 1.56 | LEU | 54 | ICL1 | 58% | 0% |
| | | | | | | | | **ALA** | **441** | **7.54** | **PHE** | **447** | **Cter** | **84%** | **79%** |
| | | | | | | | | **ASP** | **97** | **3.26** | **ARG** | **169** | **ECL2** | **85%** | **72%** |
| ASN | 43 | 1.50 | SER | 411 | 7.46 | 0% | 58% | ASN | 41 | 1.50 | SER | 433 | 7.46 | 46% | 52% |
| | | | | | | | | **SER** | **76** | **2.57** | **TYR** | **430** | **7.43** | **73%** | **79%** |
| | | | | | | | | **GLN** | **163** | **ECL2** | **ARG** | **169** | **ECL2** | **64%** | **88%** |
| **SER** | **36** | **1.43** | **MET** | **79** | **2.58** | **77%** | **73%** | | | | | | | | |
| LEU | 419 | 7.54 | ARG | 426 | Cter | 0% | 61% | ALA | 441 | 7.54 | LYS | 448 | Cter | 24% | 61% |
| | | | | | | | | **TRP** | **400** | **6.48** | **CYS** | **429** | **7.42** | **80%** | **65%** |
| | | | | | | | | **THR** | **399** | **6.47** | **LEU** | **428** | **7.41** | **75%** | **66%** |
| | | | | | | | | **MET** | **45** | **1.54** | **PHE** | **63** | **2.44** | **80%** | **59%** |
| | | | | | | | | **ASP** | **120** | **3.49** | **ARG** | **135** | **ICL2** | **80%** | **58%** |
| **GLN** | **165** | **ecl2** | **ARG** | **171** | **ecl2** | **65%** | **70%** | | | | | | | | |
| | | | | | | | | **GLN** | **163** | **ecl2** | **VAL** | **168** | **ecl2** | **63%** | **70%** |
| SER | 184 | 5.34 | THR | 390 | 6.59 | 64% | 0% | SER | 182 | 5.34 | THR | 411 | 6.59 | 0% | 63% |
| | | | | | | | | **LEU** | **442** | **7.55** | **LYS** | **448** | **Cter** | **67%** | **55%** |
| | | | | | | | | **MET** | **45** | **1.54** | **ALA** | **66** | **2.47** | **59%** | **61%** |
| ASN | 60 | 2.39 | ARG | 136 | ICL2 | 0% | 53% | ASN | 58 | 2.39 | ARG | 135 | ICL2 | 57% | 9% |
| | | | | | | | | **MET** | **45** | **1.54** | **CYS** | **67** | **2.48** | **57%** | **61%** |
| | | | | | | | | **ILE** | **48** | **1.57** | **ASN** | **59** | **2.40** | **46%** | **67%** |
| | | | | | | | | **TYR** | **80** | **2.61** | **TRP** | **99** | **3.28** | **51%** | **56%** |
| | | | | | | | | **TRP** | **400** | **6.48** | **ASN** | **432** | **7.45** | **44%** | **62%** |
| | | | | | | | | **SER** | **107** | **3.36** | **TRP** | **400** | **6.48** | **45%** | **59%** |
| | | | | | | | | **TYR** | **80** | **2.61** | **TRP** | **427** | **7.40** | **49%** | **54%** |
| | | | | | | | | **THR** | **187** | **5.39** | **VAL** | **407** | **6.55** | **49%** | **51%** |
| | | | | | | | | **ASN** | **41** | **1.50** | **LEU** | **70** | **2.51** | **48%** | **51%** |
| | | | | | | | | **ILE** | **48** | **1.57** | **GLN** | **55** | **ICL1** | **74%** | **5%** |
| | | | | | | | | **VAL** | **44** | **1.53** | **ALA** | **441** | **7.54** | **51%** | **27%** |
| | | | | | | | | **GLN** | **55** | **ICL1** | **TYR** | **60** | **2.41** | **55%** | **20%** |
| | | | | | | | | **SER** | **16** | **Nter** | **ILE** | **86** | **ECL1** | **22%** | **50%** |

**Table S2:** Intra-receptor noncovalent interactions in the four receptor—G-protein complexes. Residue names and numbers are followed by their Ballesteros-Weinstein numbers [1] and % of 2 μs simulation time that those interactions are observed.

**References**


1. Ballesteros, J.A.; Weinstein, H. Integrated methods for the construction of threedimensional models and computational probing of structure-function relations in G protein-coupled receptors. In *Methods in Neurosciences*, Sealfon, S.C., Ed. Academic: 1995; Vol. 25, pp. 366-428.
2. Flock, T.; Ravarani, C.N.J.; Sun, D.; Venkatakrishnan, A.J.; Kayikci, M.; Tate, C.G.; Veprintsev, D.B.; Babu, M.M. Universal allosteric mechanism for Galpha activation by GPCRs. *Nature* **2015**, *524*, 173-179, doi:10.1038/nature14663.