**Supplementary Information Table 1.**

**Cryo-EM data collection, refinement and validation statistics**

|  |  |  |
| --- | --- | --- |
|  | #1 name(EMDB EMD-8978)(PDB 6E3Y) |  |
| **Data collection and processing** |  |  |
| Magnification  | 47170 |  |
| Voltage (kV) | 300 |  |
| Electron exposure (e–/Å2) | 50 |  |
| Defocus range (μm) | -0.6 |  |
| Pixel size (Å) | 1.06 |  |
| Symmetry imposed | C1 |  |
| Initial particle images (no.) | 1,205,000 |  |
| Final particle images (no.) | 407,000 |  |
| Map resolution (Å) FSC threshold  | 3.260.143 |  |
| Map resolution range (Å) | 3.0-3.8 |  |
|  |  |  |
| **Refinement** |  |  |
| Initial model used (PDB code) | 5UZ7, 4RWG, 6B3J |  |
| Model resolution (Å) FSC threshold | 0.143 |  |
| Model resolution range (Å) | 3.26 |  |
| Map sharpening *B* factor (Å2) | -50 |  |
| Model composition Non-hydrogen atoms Protein residues Ligands | 1195 |  |
| *B* factors (Å2) Protein Ligand | 93-229 (avr. 148) |  |
| R.m.s. deviations Bond lengths (Å) Bond angles (°) | 0.0070.999 |  |
|  Validation MolProbity score Clashscore Poor rotamers (%)  | 1.344.510 |  |
|  Ramachandran plot Favored (%) Allowed (%) Disallowed (%) | 97.412.590 |  |

**Supplementary Information Table 2. Hydrogen bonds and contacts formed between RAMP1 and CLR during molecular dynamics simulations.** Persistence is defined as following: (total number of hydrogen bonds between two given residues / total number of frames) \* 100; a persistence > 100% is possible when more than one hydrogen bond is observed between the two residues in a given frame. Hydrogen bonds with persistence > **1%** and contacts with persistence **> 20%** are shown. If not specified, a side chain-side chain hydrogen bond is reported, otherwise **bb**=backbone-backbone hydrogen bond; **sb**=side chain-backbone; **bs**=backbone-side chain; **tb**=terminus-backbone. Interactions to ECL2 (Y2774.67-H2895.66) and ECL3 (G3466.50-V3647.37) of CLR are shown in green and blue, respectively; interactions to the CLR ECD (E29ECD-T131ECD) are shown in grey. Interactions for the alternative RAMP1 C-terminus conformation are shown in *italics* for residues S141R – V148R. CLR residues involved in hydrogen bonds with persistence greater than 25 are shown in **bold;** CLR residues involved in multiple hydrogen bonds to a given RAMP1 residue and for which the sum of the persistence is greater than 25 are shown in bold and are underlined. CLR residues involved in contacts with persistence greater than 50 are shown in **bold** and are **underlined**.

|  |  |  |
| --- | --- | --- |
| RAMP1 residue | CLR hydrogen bonds (% frames) | CLR contacts (% frames) |
| Glu29 | Arg119 34.56 | **Arg119 27.3** |
| Cys57 | **/** | **Tyr46 35.8** |
| Trp59 | **Thr43 16.66****Asn39 1.90** | Thr43 100.0Tyr46 99.5Met42 96.2 |
| Ile63 | **/** | Asn39 98.2Met42 90.0Thr43 68.5 |
| Tyr66 | **Gln45 6.57** | Gln45 100.0Met42 99.8Tyr46 99.3Tyr49 89.3 |
| Arg67 | **/** | Met42 99.8Arg38 95.0**Asn39 26.2** |
| Ala70 | **/** | Met42 95.5Gln45 77.2**Arg38 27.3** |
| Asp71 | Arg38 75.84 | **Arg38 78.6** |
| Phe83 | **/** | Arg119 91.9**Ser117 34.5** |
| Trp84 | **Arg119 2.38 (bs)** | **Met 42 21.4****Arg119 20.7** |
| Pro85 | **Arg119 2.39 (bs)** | Gly71 78.8Asp70 69.8**Arg119 27.1****Trp69 27.1** |
| Asn86 | **/** | **Arg119 31.5** |
| Asp90 | Tyr49 64.91 | Tyr49 100.0**Thr68 20.2** |
| Phe93 | **/** | Tyr49 99.2Gln45 89.5 |
| Leu94 | **/** | Ile52 99.8Met53 99.4Tyr49 93.1 |
| His97 | Gln50 55.75 | Gln50 100.0Tyr49 100.0Tyr46 100.0Met53 95.8 |
| Gly98 | **/** | Met53 91.1 |
| Phe101 | **/** | Gln50 100.0Tyr46 99.3Met53 58.7 |
| Arg102 | Asp55 65.81 **Gln54 1.04** | Met53 92.7Asp55 64.5**Gln54 31.3** |
| Cys104 | **/** | Gln50 99.8Tyr46 69.6 |
| Pro105 | **Tyr46 15.19 (bs)****Gln50 10.49 (bs)** | Gln50 99.6 |
| Ile106 | **/** | **Tyr46 41.3** |
| Ser107 | Glu47 25.19 | Glu47 94.9**Lys51 35.8** |
| Gly108 | **Glu47 23.90 (bs)** | Glu47 87.9 |
| Arg109 | **/** | Thr43 88.3Tyr46 75.3Glu47 67.8 |
| Ala110 | **Glu47 24.24 (bs)** | Thr43 90.0Glu47 67.6 |
| Val111 | **/** | Tyr278 89.5**Tyr277 40.2****Lys40 22.7** |
| Arg112 | Glu47 101.94**Asp90 10.80** | Glu47 83.4Tyr278 72.0**Tyr277 41.8** |
| Asp113 | Thr288 49.20**His289 33.99 (sb)**Tyr278 32.81 **His289 18.00** | His289 86.3Tyr278 73.6Thr288 72.3**Leu290 47.7****Tyr277 21.7** |
| Pro114 | **/** | Tyr277 91.1Leu290 85.1**Tyr278 35.4** |
| Ile118 | **/** | **Tyr277 53.0** |
| Leu119 | **/** | His289 93.6Leu290 80.1**Ile293 28.4** |
| Phe122 | **/** | Ile293 94.7Ile269 82.7Ala273 71.7Tyr277 54.1**Leu290 35.5****Leu276 23.4****Ile272 22.3** |
| Ile123 | **/** | Tyr292 99.6Ile293 92.1His289 55.5 |
| Pro126 | **/** | Pro297 98.5Ile293 96.8Pro266 83.6Ile269 68.5 |
| Ile127 |  **/** | Gly296 98.4Pro297 97.1Ala300 95.7 |
| Val129 | **/** | Phe262 97.5 |
| Thr130 | **/** | Phe262 99.8Pro297 99.6Phe228 97.6Ala300 72.2**Ala301 30.5** |
| Leu131 | **/** | Ala300 78.0Val304 66.4 |
| Val133 | **/** | Leu258 99.0Phe262 90.1Phe257 67.9 |
| Thr134 | **/** | Leu231 98.9Ile235 98.8Val302 95.6Leu258 68.5 |
| Leu136 | **/** | Trp254 67.9 |
| Val137 | **/** | Trp254 99.4Leu258 98.3Ile235 95.6Tyr255 88.8**Phe257 26.5** |
| Val138 | **/** | Ile235 99.2Phe308 86.1**His238 22.5** |
| Gln140 | **/** | Trp254 98.0**His251 22.2** |
| Ser141 | **Tyr255 22.72** **Thr239 6.24*****Thr239 12.37 (bs)******Tyr255 8.55******Thr239 6.54******Gln250 6.19 (bs)******Tyr255 2.97*** | Thr239 91.2Tyr255 86.6Ile235 75.1Gln250 54.9**His251 29.4***Tyr255 95.5**Thr239 92.3**Ile235 84.5**Gln250 69.0* |
| Lys142 |  **Glu248 1.47** | Val243 92.1Thr239 66.0Ala244 50.5*Val243 89.8**Ala244 78.8**Thr239 72.4* |
| Arg143 | **Gln250 1.15 (bs)*****Gln250 1.41 (bs)*** | **/*****Gln250 34.7******His251 20.4*** |
| Thr144 | **Lys249 2.35 (sb)*****Lys249 1.67 (bs)*** | **Lys249 35.2****Ala247 34.7****Phe246 22.0****Gln250 21.1*****Ala247 49.3******Phe246 42.2******Gln250 39.5******Ala244 35.5******Lys249 28.1*** |
| Glu145 | **Lys 249 4.61*****Lys249 3.76******Lys249 3.66 (bb)*** | **Lys249 23.7****His251 20.2*****Gln250 41.4******Lys249 39.0******His251 35.8*** |
| Gly146 | **Trp254 2.24 (bs)*****His251 1.62 (sb)*** | **Lys249 22.4*****His251 24.2*** |
| Ile147 | **/*****/*** | **His251 26.3*****His251 20.9*** |
| Val148 | **Lys249 3.75****His251 2.89 (sb)*****Lys249 2.19*** | **His251 24.5** |

**Supplementary Information Table 3.** **Hydrogen bonds and contacts formed between CGRP and CLR/RAMP1 during molecular dynamics simulations.** Persistence is defined as the (total number of hydrogen bonds between two given residues / total number of frames) \* 100; a persistence > 100% is possible when more than one hydrogen bond is observed between the two residues in a given frame. Hydrogen bonds with persistence > **1%** and contacts with persistence **> 20%** are shown. If not specified, a side chain-side chain hydrogen bond is reported, otherwise **bb**=backbone-backbone hydrogen bond; **sb**=side chain-backbone; **bs**=backbone-side chain; **tb**=terminus-backbone. Interactions to ECL1 (T1962.69-P2093.74), ECL2 (Y2774.67-H2895.66) and ECL3 (G3466.50-V3647.37) of CLR are shown in red, green and blue respectively; interactions to the CLR ECD (Q33ECD-T131ECD) are shown in grey. CLR residues involved in multiple hydrogen bonds to a given CGRP residue and for which the sum of the persistence is greater than 25 are shown in **bold** and are **underlined**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| CGRP residue | CLR hydrogen bonds (% frames) | CLR contacts (% frames) | RAMP1 hydrogen bonds (% frames) | RAMP1 contacts (% frames) |
| A1 | **Asp287 3.76 (bs)****His289 3.71 (bs)****Asp366 2.59 (bs)** | Asp287 52.4**Ser286 37.1****Tyr292 34.7****His289 27.8****Arg355 27.5** | **/** | **/** |
| C2 | **Arg355 1.29 (bs)** | Ser286 65.7Leu291 63.4**Tyr292 47.1****His295 24.2** | **/** | **/** |
| D3 | Arg355 34.71**Tyr292 7.50****Lys359 3.78** | Arg355 79.0Trp354 62.4**Tyr292 42.5** | **/** | **/** |
| T4 | **His295 1.19** | His295 74.0**Trp354 47.8****Cys299 35.0****Tyr292 26.8** | **/** | **/** |
| A5 | **/** | Phe349 84.5Met373 62.6Met369 57.4**Trp354 42.2****Tyr227 21.9** | **/** | **/** |
| T6 | His295 31.13 | His295 81.5Ile298 74.6Tyr227 70.5Met223 68.8**Met373 38.2****Phe349 35.9****Leu302 27.0** | **/** | **/** |
| C7 | **/** | His295 60.3Leu291 60.3**Ser286 45.6** | **/** | **/** |
| V8 | **/** | His370 96.4Met373 95.9Met369 71.0**His374 33.7****Trp354 24.6** | **/** | **/** |
| T9 | **His219 18.98****His374 2.16** | Thr191 92.7Leu195 87.0His219 76.9His194 59.6Met373 52.7His374 51.6**Met223 37.2** | **/** | **/** |
| H10 | **His295 7.46****Arg274 2.48** | Ser286 91.1Ile284 69.8Leu220 69.3Leu291 69.2Gln216 69.1His219 62.9**His295 44.7****His194 43.3****Trp283 26.2****Met223 23.9****Ser285 21.7** | **/** | **/** |
| R11 | Asp366 85.37**Asp96 8.37****Asp287 6.24** | Ser286 73.6Asp366 71.3His370 61.6**Trp354 25.1** | **/** | **/** |
| L12 | **/** | Leu141 97.4Leu195 95.4Thr145 85.3His370 83.6Phe142 65.9His374 61.3**Ala138 43.0** | **/** | **/** |
| A13 | **/** | Leu195 95.2Ile284 90.8His194 71.9Ala199 54.5**Val198 49.9** | **/** | **/** |
| G14 | **/** | **Ile284 77.0****Ser286 22.5** | **/** | **/** |
| L15 | **/** | **Ala138 99.2****Lys134 67.3****Leu141 48.5** | **/** | **/** |
| L16 | **/** | Phe142 99.6Ala199 98.2Leu195 90.3Ala138 74.1Leu139 58.1**Val198 38.9****Asn200 36.7** | **/** | **/** |
| S17 | **Gln202 2.95 (bs)** | Ile284 68.2Gln202 63.4Ala199 59.4**Val198 47.5****Leu204 32.0****Val205 28.8** | **/** | **/** |
| R18 | **Asp287 22.96****Asp90 4.47****Asp96 4.31** | Pro97 92.2Gln93 87.6Phe95 52.5Asp96 51.7**Asp90 34.8****Asp287 23.9** | **/** | **/** |
| S19 | **Gln93 7.74 (sb)** | Val135 99.5Ala138 75.9**Leu139 28.1** | **/** | **/** |
| G20 | **/** | **Gln202 68.7** | **/** | **/** |
| G21 | **/** | Gln93 78.2Gln202 53.8 | **/** | **/** |
| V22 | **/** | Val135 91.3Asp94 90.1Gln93 87.5Thr131 79.3**His132 36.8** | **/** | **/** |
| V23 | **/** | Val135 83.8Leu139 60.0 | **/** | **/** |
| K24 | **Gln202 1.45** | Gln202 65.2 | **/** | **/** |
| N25 | **/** | **/** | **/** | **/** |
| N26 | **/** | **/** | **/** | **/** |
| F27 | **/** | Asp94 90.8Gln93 73.7 | **/** | **/** |
| V28 | **/** | **/** | **/** | **/** |
| P29 | **/** | Asp94 89.2**Asn128 21.6** | **/** | **/** |
| T30 | Asp94 62.32Asp94 56.01 **(bs)** | Asp94 96.7Asn128 93.4Phe95 93.2Phe92 92.9Trp72 75.6 | **/** | **/** |
| N31 | **/** | Trp72 77.6 | **/** | **/** |
| V32 | **/** | Tyr124 93.2Phe95 92.2Thr125 90.5Asn128 88.1Trp72 67.1Trp121 50.6 | **/** | **/** |
| G33 | **Trp121 17.80 (bs)** | Trp121 55.9 | **/** | **/** |
| S34 | **Arg119 6.20 (bs)****Ser117 3.66 (bs)****His114 3.33** | Trp121 51.4**His114 49.7****Arg119 43.0****Ser117 40.1****Ala116 21.1** | **Trp84 1.36** | **Phe83 26.5** |
| K35 | **Arg119 1.07 (bs)** | **Arg119 34.4** | **Glu78 7.77** | Phe83 55.5 |
| A36 | **R119 6.04 (bs)** | Trp72 54.6Trp121 50.2**Arg119 28.6** | **/** | **/** |
| F37 | **T122 11.48 (tb)****T122 8.22 (bb)****Asp70 1.62**  | Trp72 89.4Gly71 79.2Tyr124 55.6Asp70 52.7**Thr122 34.5****Trp121 30.1****Arg119 22.8** | **/** | Trp84 87.9Pro85 78.9Trp74 57.7**Phe83 22.4** |

**Supplementary Information Table 4.** **The difference in hydrogen bond formation between CGRP and CLR, during MD simulations performed on the CGRP-CLR-Gα (371-394) complex in the presence and absence of RAMP1.** Hydrogen bond persistence is expressed as percentage on the total duration of the simulations (2.0 μs for each system). Hydrogen bonds with persistence > **5%** are shown: **sb**=side chain-backbone; **bs**=backbone-side chain; **ts**=terminus-side chain. If not specified, a side chain-side chain hydrogen bond is reported. A persistence > 100% is possible when more than one hydrogen bond is possible between the two residues. The persistence of hydrogen bonds involving Asp3, Thr6, Thr9, His10 (and Phe37) are unchanged upon loss of RAMP1, consistent with the low RMSFs in this region. The main loss of interactions is in the C-terminus; this is consistent with the higher RMSF for this region. Moreover, this may affect the proposed two-stage binding mechanism for class B peptide ligandsS1 in which the initial binding involves the C-terminus.

|  |  |  |
| --- | --- | --- |
| CGRP residue | Hydrogen bond persistence (% frames)  | Variation |
|  | With RAMP1 | Without RAMP1 |  |
| Ala1  | H289ECL2 17.4 (ts)  | H289ECL2 10.0 | ↓ |
| Arg11 | D366ECL3 108.6 | D366ECL3 134.0 | ↑↑ |
| Arg18 | D287ECL2 72.9D90ECD 14.9 | D287ECL2 45.1/ | ↓↓↓↓ |
| Ser19 | Q93ECD 7.1 (sb) | / | ↓ |
| Thr30 | D94ECD 61.1D94ECD 54.7 (bs) | D94ECD 31.0D94ECD 27.5 (bs) | ↓↓↓↓↓↓ |

Persistence change: ↑↑= 15-30% increase; ↓= 5-15% decrease; ↓↓= 15-30%; ↓↓↓= 30+% decrease. Details of the CLR – CGRP and RAMP1 – CLR interactions are available from the University of Essex Research Repository (doi to be provided).

**Supplementary Information Table 5.** **The difference in CLR intra-molecular hydrogen bonds formation** **in the presence or absence of RAMP1.** Persistence during MD simulations performed on the CGRP-CLR-Gα(371-394) complex in the presence and absence of RAMP1. Hydrogen bond persistence is expressed as percentage on the total duration of the simulations (2.0 μs for each system). **sb**=side chain-backbone; **bs**=backbone-side chain. If not specified, a side chain-side hydrogen bond is reported. A persistence > 100% is possible when more than one hydrogen bonds are possible between the two residues.

|  |  |  |
| --- | --- | --- |
| CLR intra-molecular Hydrogen bond | Hydrogen bond persistence(% frames) | Variation |
| With RAMP1 | Without RAMP1 |
| R1732.46 - E2333.50 | 214.5 | 125.2 | ↓↓↓↓ |
| R2744.64 - D280ECL2 | 157.4 | 5.8 | ↓↓↓↓↓ |
| K1341.32 - D96ECDK1341.32 - A1381.36 (sb)K1341.32 - N1301.28 (sb)K1341.32 - E99ECD | 70.463.536.732.9 | 38.049.829.99.7 | ↓↓↓↓↓↓↓ |
| D67ECD - D77ECDD67ECD - D77ECD (bs)D67ECD - K51ECD (sb) | 58.446.916.1 | 77.532.139.1 | ↑↑↓↑↑ |
| D108ECD - R113ECD | 37.6 | 79.2 | ↑↑↑ |

Persistence change: ↑↑= 15-30% increase; ↑↑↑= 30-50% increase; ↑↑↑↑= 50-100%% increase; ↓= 5-15% decrease; ↓↓= 15-30%; ↓↓↓= 30-50% decrease; ↓↓↓↓= 50-100% decrease; ↓↓↓↓↓=>100% decrease. Values over 100% arise through multiple hydrogen bonds.

**Supplementary Information Table 6. Summary of all the MD simulations performed on the CLR-CGRP-RAMP1-G-protein.** CLR conformation #4 is the original PLOP-derived conformation; CLR conformations #0-3 were taken from the 4 highest occupied clusters. αβγ denotes the full G-protein, while α371-394denotesthe C terminal helix αH5 (N371 - L394) of the G-protein α subunit.

|  |  |  |  |
| --- | --- | --- | --- |
| Conformation | G protein | Number of replicas | Total MD sampling time |
| ECL3 | RAMP1 C-term |
| #0 | #1 | αβγ | 4  | 1.6 μs |
| #1 | #1 | αβγ | 4 | 1.6 μs |
| #2 | #1 | αβγ | 4 | 1.6 μs |
| #3 | #1 | αβγ | 4 | 1.6 μs |
| #1 | #2 | αβγ | 10 | 2.4 μs |
| ***Total CLR:CGRP:RAMP1:αβγ simulation time*** | ***8.8 μs*** |
| #4 | #1 | (α371-394) | 4 | 2.0 μs |
| #4 | N/A | (α371-394) | 4 | 2.0 μs |

**Supporting Information specific references**

S1. de Graaf, C., *et al.,* Extending the structural view of class B GPCRs. *Trends Biochem Sci* **42**, 946−960 (2017).