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Cryo-EM structure of the active, G_s-protein complexed, human CGRP receptor

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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 ⁻ /Å ²)	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)	3.26
FSC threshold	0.143
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 <i>B</i> factor (Å ²)	-50
Model composition	
Non-hydrogen atoms	
Protein residues	1195
Ligands	
<i>B</i> factors (Å ²)	93-229 (avr. 148)
Protein	
Ligand	
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	0.999
Validation	
MolProbity score	1.34
Clashscore	4.51
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	97.41
Allowed (%)	2.59
Disallowed (%)	0

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 (Y277^{4.67}-H289^{5.66}) and ECL3 (G346^{6.50}-V364^{7.37}) of CLR are shown in green and blue, respectively; interactions to the CLR ECD (E29^{ECD}-T131^{ECD}) are shown in grey. Interactions for the alternative RAMP1 C-terminus conformation are shown in *italics* for residues S141^R – V148^R. 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	<u>Arg119 34.56</u>	Arg119 27.3
Cys57	/	Tyr46 35.8
Trp59	Thr43 16.66 Asn39 1.90	Thr43 100.0 Tyr46 99.5 Met42 96.2
Ile63	/	Asn39 98.2 Met42 90.0 Thr43 68.5
Tyr66	Gln45 6.57	Gln45 100.0 Met42 99.8 Tyr46 99.3 Tyr49 89.3
Arg67	/	Met42 99.8 Arg38 95.0 Asn39 26.2
Ala70	/	Met42 95.5 Gln45 77.2 Arg38 27.3
Asp71	<u>Arg38 75.84</u>	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 78.8 Asp70 69.8 Arg119 27.1 Trp69 27.1
Asn86	/	Arg119 31.5
Asp90	Tyr49 64.91	Tyr49 100.0 Thr68 20.2
Phe93	/	Tyr49 99.2 Gln45 89.5

Leu94	/	Ile52 99.8 Met53 99.4 Tyr49 93.1
His97	Gln50 55.75	Gln50 100.0 Tyr49 100.0 Tyr46 100.0 Met53 95.8
Gly98	/	Met53 91.1
Phe101	/	Gln50 100.0 Tyr46 99.3 Met53 58.7
Arg102	<u>Asp55 65.81</u> Gln54 1.04	Met53 92.7 Asp55 64.5 Gln54 31.3
Cys104	/	Gln50 99.8 Tyr46 69.6
Pro105	Tyr46 15.19 (bs) Gln50 10.49 (bs)	Gln50 99.6
Ile106	/	Tyr46 41.3
Ser107	<u>Glu47 25.19</u>	Glu47 94.9 Lys51 35.8
Gly108	Glu47 23.90 (bs)	Glu47 87.9
Arg109	/	Thr43 88.3 Tyr46 75.3 Glu47 67.8
Ala110	Glu47 24.24 (bs)	Thr43 90.0 Glu47 67.6
Val111	/	Tyr278 89.5 Tyr277 40.2 Lys40 22.7
Arg112	<u>Glu47 101.94</u> Asp90 10.80	Glu47 83.4 Tyr278 72.0 Tyr277 41.8
Asp113	Thr288 49.20 His289 33.99 (sb) Tyr278 32.81 His289 18.00	His289 86.3 Tyr278 73.6 Thr288 72.3 Leu290 47.7 Tyr277 21.7
Pro114	/	Tyr277 91.1 Leu290 85.1 Tyr278 35.4
Ile118	/	Tyr277 53.0
Leu119	/	His289 93.6 Leu290 80.1 Ile293 28.4
Phe122	/	Ile293 94.7 Ile269 82.7 Ala273 71.7 Tyr277 54.1 Leu290 35.5 Leu276 23.4 Ile272 22.3

Ile123	/	Tyr292 99.6 Ile293 92.1 His289 55.5
Pro126	/	Pro297 98.5 Ile293 96.8 Pro266 83.6 Ile269 68.5
Ile127	/	Gly296 98.4 Pro297 97.1 Ala300 95.7
Val129	/	Phe262 97.5
Thr130	/	Phe262 99.8 Pro297 99.6 Phe228 97.6 Ala300 72.2 Ala301 30.5
Leu131	/	Ala300 78.0 Val304 66.4
Val133	/	Leu258 99.0 Phe262 90.1 Phe257 67.9
Thr134	/	Leu231 98.9 Ile235 98.8 Val302 95.6 Leu258 68.5
Leu136	/	Trp254 67.9
Val137	/	Trp254 99.4 Leu258 98.3 Ile235 95.6 Tyr255 88.8 Phe257 26.5
Val138	/	Ile235 99.2 Phe308 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 91.2 Tyr255 86.6 Ile235 75.1 Gln250 54.9 His251 29.4 Tyr255 95.5 Thr239 92.3 Ile235 84.5 Gln250 69.0
Lys142	Glu248 1.47	Val243 92.1 Thr239 66.0 Ala244 50.5 Val243 89.8

		<i>Ala244 78.8</i> <i>Thr239 72.4</i>
Arg143	Gln250 1.15 (bs) <i>Gln250 1.41 (bs)</i>	<i>/</i> <i>Gln250 34.7</i> <i>His251 20.4</i>
Thr144	Lys249 2.35 (sb) <i>Lys249 1.67 (bs)</i>	Lys249 35.2 Ala247 34.7 Phe246 22.0 Gln250 21.1 <i>Ala247 49.3</i> <i>Phe246 42.2</i> <i>Gln250 39.5</i> <i>Ala244 35.5</i> <i>Lys249 28.1</i>
Glu145	Lys 249 4.61 <i>Lys249 3.76</i> <i>Lys249 3.66 (bb)</i>	Lys249 23.7 His251 20.2 <i>Gln250 41.4</i> <i>Lys249 39.0</i> <i>His251 35.8</i>
Gly146	Trp254 2.24 (bs) <i>His251 1.62 (sb)</i>	Lys249 22.4 <i>His251 24.2</i>
Ile147	<i>/</i> <i>/</i>	His251 26.3 <i>His251 20.9</i>
Val148	Lys249 3.75 His251 2.89 (sb) <i>Lys249 2.19</i>	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 (T196^{2.69}-P209^{3.74}), ECL2 (Y277^{4.67}-H289^{5.66}) and ECL3 (G346^{6.50}-V364^{7.37}) of CLR are shown in **red**, **green** and **blue** respectively; interactions to the CLR ECD (Q33^{ECD}-T131^{ECD}) 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 65.7 Leu291 63.4 Tyr292 47.1 His295 24.2	/	/
D3	Arg355 34.71 Tyr292 7.50 Lys359 3.78	Arg355 79.0 Trp354 62.4 Tyr292 42.5	/	/
T4	His295 1.19	His295 74.0 Trp354 47.8 Cys299 35.0 Tyr292 26.8	/	/
A5	/	Phe349 84.5 Met373 62.6 Met369 57.4 Trp354 42.2 Tyr227 21.9	/	/
T6	His295 31.13	His295 81.5 Ile298 74.6 Tyr227 70.5 Met223 68.8 Met373 38.2 Phe349 35.9 Leu302 27.0	/	/
C7	/	His295 60.3 Leu291 60.3 Ser286 45.6	/	/
V8	/	His370 96.4 Met373 95.9	/	/

		Met369 71.0 His374 33.7 Trp354 24.6		
T9	His219 18.98 His374 2.16	Thr191 92.7 Leu195 87.0 His219 76.9 His194 59.6 Met373 52.7 His374 51.6 Met223 37.2	/	/
H10	His295 7.46 Arg274 2.48	Ser286 91.1 Ile284 69.8 Leu220 69.3 Leu291 69.2 Gln216 69.1 His219 62.9 His295 44.7 His194 43.3 Trp283 26.2 Met223 23.9 Ser285 21.7	/	/
R11	<u>Asp366 85.37</u> Asp96 8.37 Asp287 6.24	Ser286 73.6 Asp366 71.3 His370 61.6 Trp354 25.1	/	/
L12	/	Leu141 97.4 Leu195 95.4 Thr145 85.3 His370 83.6 Phe142 65.9 His374 61.3 Ala138 43.0	/	/
A13	/	Leu195 95.2 Ile284 90.8 His194 71.9 Ala199 54.5 Val198 49.9	/	/
G14	/	Ile284 77.0 Ser286 22.5	/	/
L15	/	Ala138 99.2 Lys134 67.3 Leu141 48.5	/	/
L16	/	Phe142 99.6 Ala199 98.2 Leu195 90.3 Ala138 74.1 Leu139 58.1 Val198 38.9 Asn200 36.7	/	/
S17	Gln202 2.95 (bs)	Ile284 68.2 Gln202 63.4 Ala199 59.4	/	/

		Val198 47.5 Leu204 32.0 Val205 28.8		
R18	Asp287 22.96 Asp90 4.47 Asp96 4.31	Pro97 92.2 Gln93 87.6 Phe95 52.5 Asp96 51.7 Asp90 34.8 Asp287 23.9	/	/
S19	Gln93 7.74 (sb)	Val135 99.5 Ala138 75.9 Leu139 28.1	/	/
G20	/	Gln202 68.7	/	/
G21	/	Gln93 78.2 Gln202 53.8	/	/
V22	/	Val135 91.3 Asp94 90.1 Gln93 87.5 Thr131 79.3 His132 36.8	/	/
V23	/	Val135 83.8 Leu139 60.0	/	/
K24	Gln202 1.45	Gln202 65.2	/	/
N25	/	/	/	/
N26	/	/	/	/
F27	/	Asp94 90.8 Gln93 73.7	/	/
V28	/	/	/	/
P29	/	Asp94 89.2 Asn128 21.6	/	/
T30	<u>Asp94 62.32</u> <u>Asp94 56.01</u> (bs)	Asp94 96.7 Asn128 93.4 Phe95 93.2 Phe92 92.9 Trp72 75.6	/	/
N31	/	Trp72 77.6	/	/
V32	/	Tyr124 93.2 Phe95 92.2 Thr125 90.5 Asn128 88.1 Trp72 67.1 Trp121 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 54.6	/	/

		Trp121 50.2 Arg119 28.6		
F37	T122 11.48 (tb) T122 8.22 (bb) Asp70 1.62	Trp72 89.4 Gly71 79.2 Tyr124 55.6 Asp70 52.7 Thr122 34.5 Trp121 30.1 Arg119 22.8	/	Trp84 87.9 Pro85 78.9 Trp74 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-G α (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 μ s 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 ligands^{S1} in which the initial binding involves the C-terminus.

CGRP residue	Hydrogen bond persistence (% frames)				Variation
	With RAMP1		Without RAMP1		
Ala ¹	H289 ^{ECL2}	17.4 (ts)	H289 ^{ECL2}	10.0	↓
Arg ¹¹	D366 ^{ECL3}	108.6	D366 ^{ECL3}	134.0	↑↑↑
Arg ¹⁸	D287 ^{ECL2}	72.9	D287 ^{ECL2}	45.1	↓↓↓
	D90 ^{ECD}	14.9	/		↓
Ser ¹⁹	Q93 ^{ECD}	7.1 (sb)	/		↓
Thr ³⁰	D94 ^{ECD}	61.1	D94 ^{ECD}	31.0	↓↓↓
	D94 ^{ECD}	54.7 (bs)	D94 ^{ECD}	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	
R173 ^{2.46} - E233 ^{3.50}	214.5	125.2	↓↓↓↓↓
R274 ^{4.64} - D280 ^{ECL2}	157.4	5.8	↓↓↓↓↓↓
K134 ^{1.32} - D96 ^{ECD}	70.4	38.0	↓↓↓
K134 ^{1.32} - A138 ^{1.36} (sb)	63.5	49.8	↓
K134 ^{1.32} - N130 ^{1.28} (sb)	36.7	29.9	↓
K134 ^{1.32} - E99 ^{ECD}	32.9	9.7	↓↓
D67 ^{ECD} - D77 ^{ECD}	58.4	77.5	↑↑
D67 ^{ECD} - D77 ^{ECD} (bs)	46.9	32.1	↓
D67 ^{ECD} - K51 ^{ECD} (sb)	16.1	39.1	↑↑
D108 ^{ECD} - R113 ^{ECD}	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. $\alpha\beta\gamma$ denotes the full G-protein, while $\alpha_{371-394}$ denotes the C terminal helix α H5 (N371 - L394) of the G-protein α subunit.

Conformation		G protein	Number of replicas	Total MD sampling time
ECL3	RAMP1 C-term			
#0	#1	$\alpha\beta\gamma$	4	1.6 μ s
#1	#1	$\alpha\beta\gamma$	4	1.6 μ s
#2	#1	$\alpha\beta\gamma$	4	1.6 μ s
#3	#1	$\alpha\beta\gamma$	4	1.6 μ s
#1	#2	$\alpha\beta\gamma$	10	2.4 μ s
Total CLR:CGRP:RAMP1:$\alpha\beta\gamma$ simulation time				8.8 μs
#4	#1	($\alpha_{371-394}$)	4	2.0 μ s
#4	N/A	($\alpha_{371-394}$)	4	2.0 μ s

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).