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Supporting Material

Structure of a Double Transmembrane Fragment of a G Protein-Coupled Receptor in Micelles

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Supplementary Material

Figure S1: Cleavage and purification of the selectively methyl labeled Ste2p(G31-T110) peptide. Top panel: Inclusion bodies containing the fusion protein prior to cleavage with CNBr. Inset: SDS-PAGE gel of the inclusion bodies to show protein expression levels stained with Coommassie Blue (M=Marker, FP=fusion protein). The arrow indicates the protein of interest with an expected MW of ~23 kDa. Middle panel: Chromatogram of CNBr cleavage reaction after 1 hour. Lower panel: Analytical RP-HPLC of the purified Ste2p(G31-T110) after CNBr cleavage and purification on a preparative scale. Analytical reversed phase HPLC was performed with a 36-90% acetonitrile:water gradient with 10% isopropanol, 0.1% trifluoroacetic acid at 60°C on a Zorbax 300SB-C3 column.

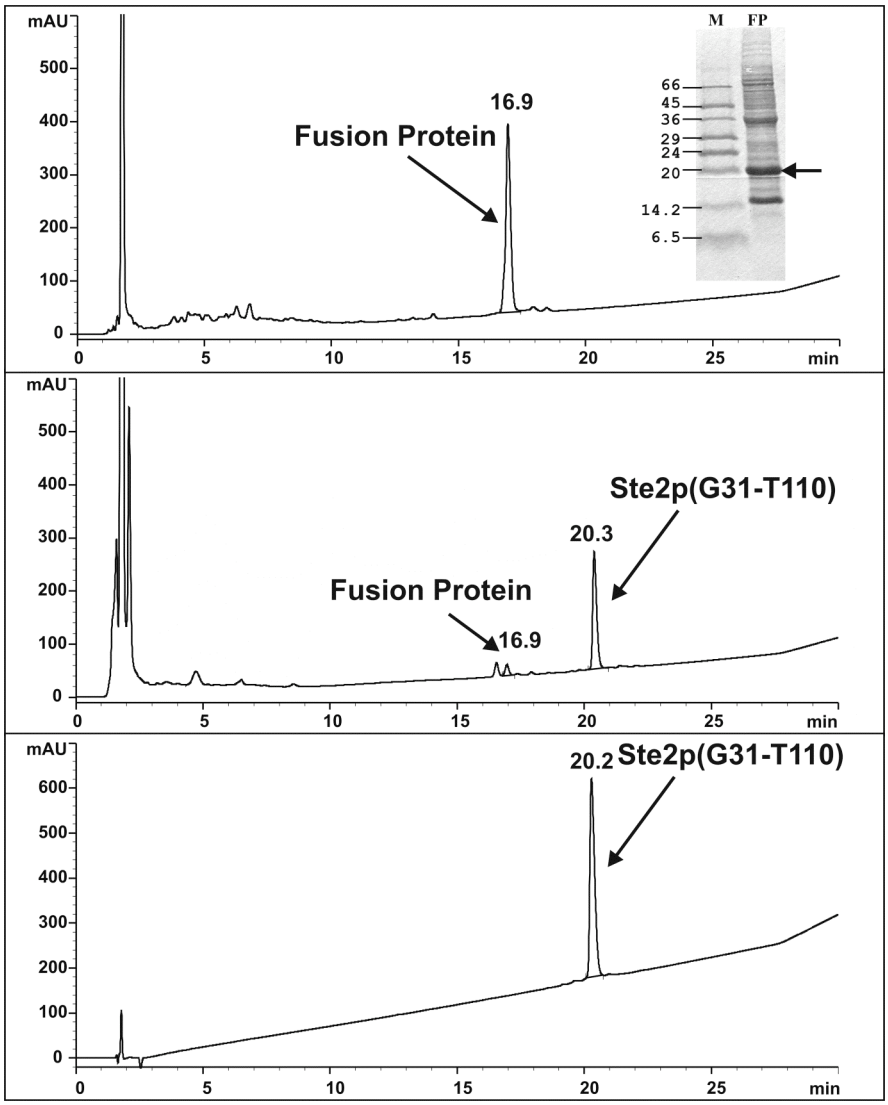

Table S2: Overview on spectroscopic details for the used 2D and 3D NMR experiments

Experiment	Data matrix size	Max. evolution times	Number of scans
[^{15}N,^{1}H]-HSQC	2048(^{1}H)*512(^{15}N)	$t_2$max 91.0ms $t_1$max 164.0ms	128
ct-[^{13}C,^{1}H]-HSQC	2048(^{1}H)*370(^{13}C)	$t_2$max 109.9ms $t_1$max 13.1ms	64
Aromatic [^{13}C,^{1}H]-HSQC	2048(^{1}H)*256(^{13}C)	$t_2$max 114.0ms $t_1$max 19.6ms	32
HNCO	2048(^{1}H)*40(^{15}N)*128(^{13}C)	$t_3$max 105ms $t_2$max 14.1ms $t_1$max 16.5ms	8
HN(CA)CO	2048(^{1}H)*40(^{15}N)*128(^{13}C)	$t_3$max 105ms $t_2$max 14.1ms $t_1$max 16.5ms	8
HNCA	2048(^{1}H)*40(^{15}N)*128(^{13}C)	$t_3$max 105ms $t_2$max 14.1ms $t_1$max 4.9ms	8
HN(CO)CA	2048(^{1}H)*40(^{15}N)*128(^{13}C)	$t_3$max 105ms $t_2$max 14.1ms $t_1$max 4.9ms	8
HNCACB	2048(^{1}H)*40(^{15}N)*128(^{13}C)	$t_3$max 105ms $t_2$max 14.1ms $t_1$max 4.9ms	16
CBCA(CO)NH	2048(^{1}H)*40(^{15}N)*128(^{13}C)	$t_3$max 105ms $t_2$max 14.1ms $t_1$max 4.9ms	16
(H)CCH-TOCSY	2048(^{1}H)*50(^{13}C)*100(^{13}C)	$t_3$max 105ms $t_2$max 4.7ms $t_1$max 9.5ms	16
^{15}N{^{1}H}-NOE	2048(^{1}H)*600(^{15}N)	$t_2$max 91.0ms $t_1$max 248.0ms	128
HMCMCBCANH	For Val and Ile residues 2048(^{1}H)*40(^{15}N)*36(^{13}C) For Leu residues 2048*40(^{15}N)*60(^{13}C)	For Val and Ile residues $t_3$max 105ms $t_2$max 12.8ms $t_1$max 6.8ms For Leu residues $t_3$max 105ms, $t_2$max 12.8ms, $t_1$max 9.5ms	64
HBCBCGCDHD	2048(^{1}H)*58(^{13}C)	$t_2$max 91ms $t_1$max 4.1ms	128
^{15}N-resolved NOESY recorded on 700MHz at University of Zurich	2048(^{1}H)*50(^{15}N)*180(^{1}H)	$t_3$max 105.0ms $t_2$max 16.0ms $t_1$max 13.0ms mixing time 100ms	32
Aliphatic ^{13}C-resolved NOESY	2048(^{1}H)*50(^{13}C)*100(^{1}H)	$t_3$max 57.0ms $t_2$max 5.1ms	16

recorded on 900MHz at New York Center for Structural Biology		$t_1$max 10.7ms mixing time 80ms	
Aromatic ^{13}C-resolved NOESY recorded on 900MHz at New York Center for Structural Biology	2048(^{1}H)*50(^{13}C)*100(^{1}H)	$t_3$max 57.0ms $t_2$max 5.1ms $t_1$max 10.7ms mixing time 80ms	16
Aliphatic-optimized ^{13}C-resolved NOESY on a selectively Me-labeled sample recorded on 900MHz at ETH, Zurich	2048(^{1}H)*50(^{13}C)*100(^{1}H)	$t_3$max 81.2ms $t_2$max 5.9ms $t_1$max 20.0ms mixing time 250ms	16

Figure S3. Strips from the 3D HNCA (left) and the ^{15}N-resolved NOESY (right) spectra of uniformly [^{15}N,^{13}C]-labeled Ste2p(G31-T110) extracted at various amide proton positions displaying the assignment and validation processes of the ^{15}N, ^{13}C and ^{1}H chemical shifts. The ^{15}N and ^{1}H chemical shifts, at which the strips were extracted, are displayed above and below the strips, respectively.

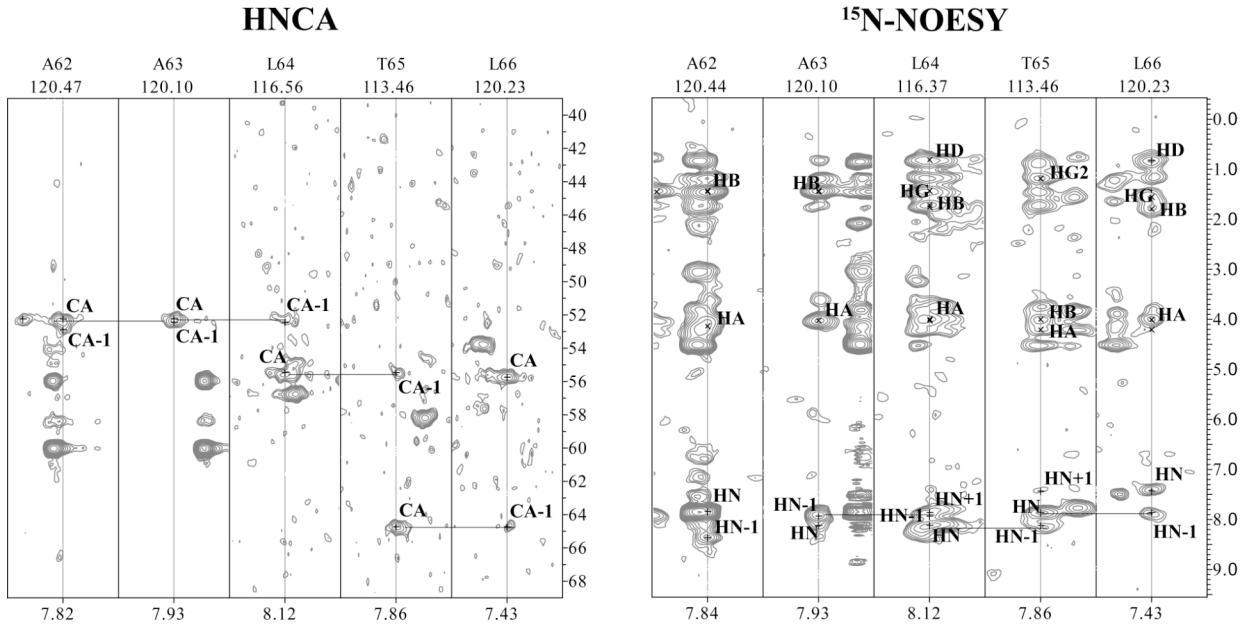

Figure S4. Methyl region from the ct-[^{13}C,^{1}H]-HSQC of various Ste2p(G31-T110) samples: Left – [^{15}N,^{13}C]-labeled Ste2p(G31-T110) in the LPPG solution, recorded on a 700MHz magnet; Middle – [^{15}N,^{13}C]-labeled Ste2p(G31-T110) in the d_{36}-LPPG solution, recorded on a 900MHz magnet; Right - [^{15}N,^{13}C,^{2}H,^{1}H(Methyl – Ile,Leu,Val)]-labeled Ste2p(G31-T110) in d_{36}-LPPG solution, recorded at 900MHz. The regions containing methyl groups of Ile or Leu residues are marked with boxes.

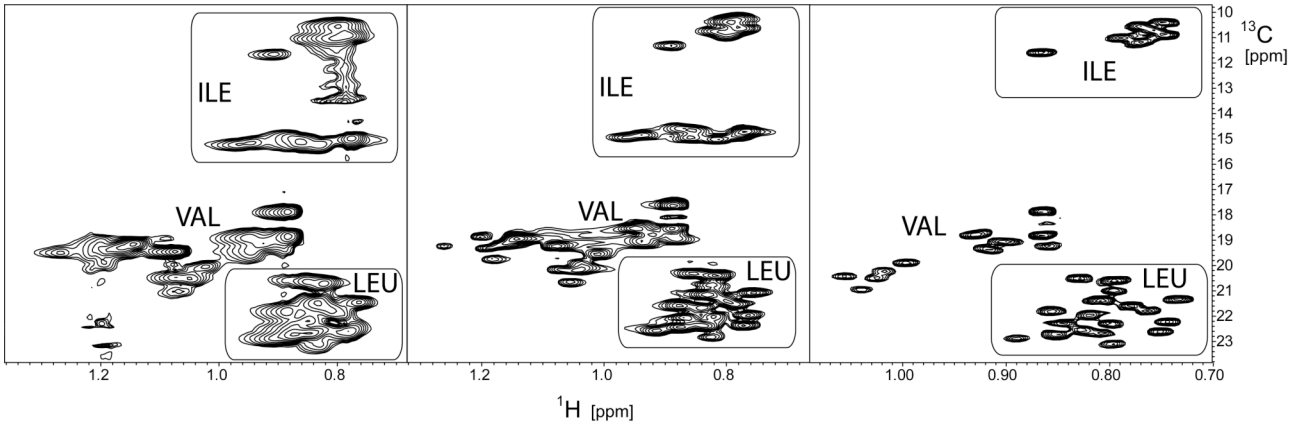

Figure S5: Values of the ^{15}N{^{1}H}-NOE of Ste2p(G31-T110). The ^{15}N{^{1}H}-NOEs were determined using spectra recorded at 700 MHz proton frequency. Highlighted with gray are the predicted α-helical regions of TM1-TM2. Residues, for which dihedral angle restraints calculated with TALOS were applied during the structure calculation, are marked by a symbol on top.

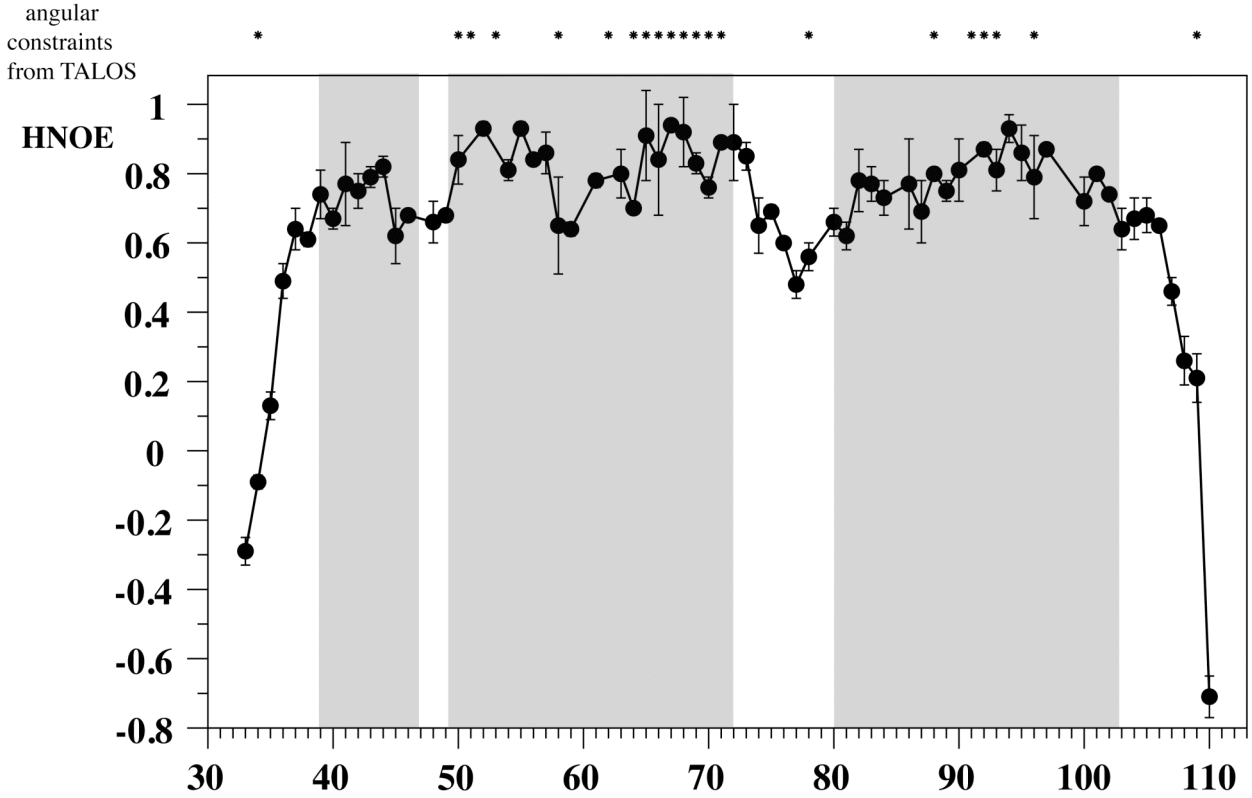

Figure S6. Superposition of the lowest-energy conformer of Ste2p(G31-T110) from the calculated 20-conformers bundle with the structure of the same region derived from homology modeling (Eilers *et al.*) fitted for backbone atoms of residues 49-72 and 80-103. Right and left representations differ by a 90º rotation about the bilayer normal. The red/yellow representation corresponds to the experimental structure and the pink/gray to the modeled structure.

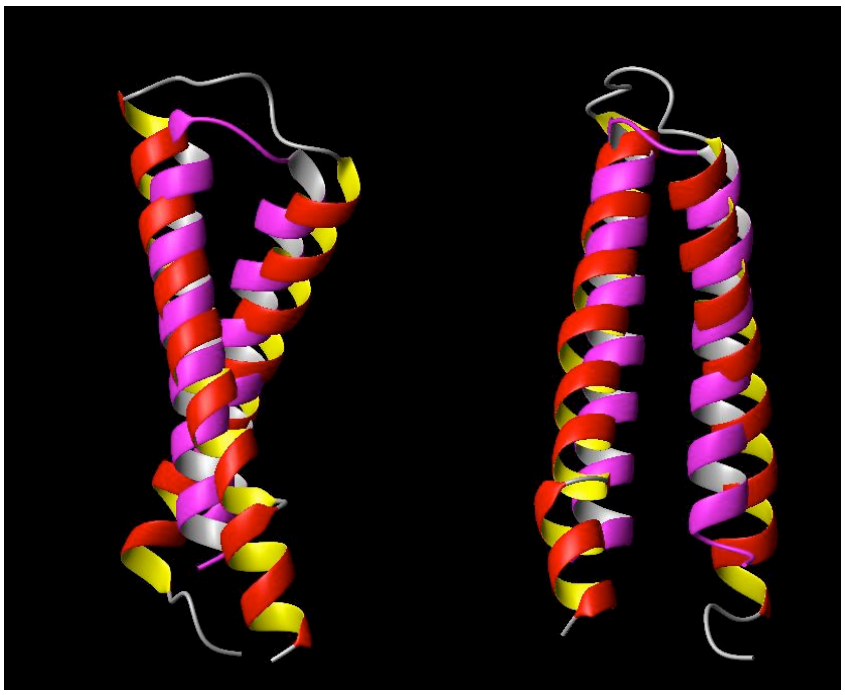

Figure S7. Relative peak volumes of signals computed from [^{15}N,^{1}H]-HSQC spectra of [^{15}N]Ste2p(G31-T110) recorded in the presence of low-power presaturation on the water resonance during the relaxation delay relative to a reference experiment without presaturation.

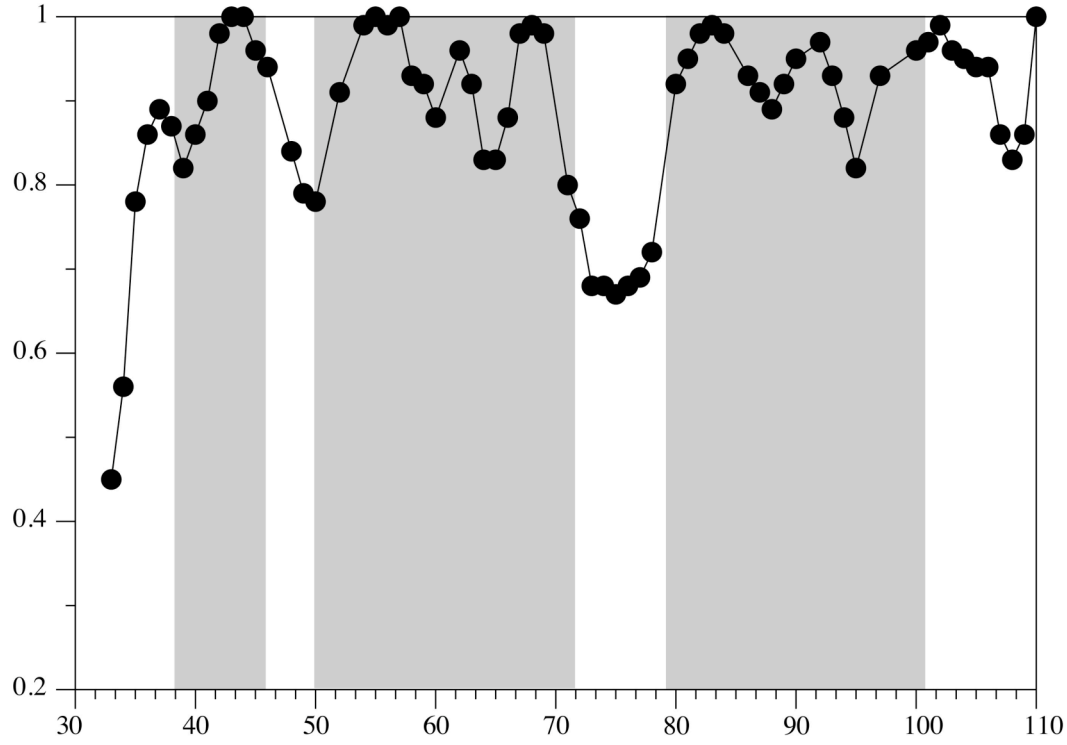

Table S8. Information on the structure calculation for Ste2p(G31-T110) in LPPG.

Distance restraints	Total	1247
	Intra-residual	439
	Sequential (i - j = 1)	378
	Medium (i - j = 2, 3, 4)	406
	Long-range	24
Dihedral angle restraints	Total	753
RMSD (Å)		
	Asp39-Ser47 backbone	0.25 ± 0.10
	Asp39-Ser47 all heavy atoms	1.32 ± 0.26
	Val49-Thr72 backbone	0.40 ± 0.13
	Val49-Thr72 all heavy atoms	1.20 ± 0.31
	Ile80-Leu103 backbone	0.57 ± 0.19
	Ile80-Leu103 all heavy atoms	1.38 ± 0.24
	Asp39-Leu103 backbone	2.36 ± 0.97
	Asp39-Leu103 all heavy atoms	3.28 ± 1.03
Structure check (Average %)		
Ramachandran statistics	most favored	83.9
(Gly31-Thr110)	additionally allowed	15.2
	generously allowed	0.9
	disallowed	0
NOE constraint violations	Number > 0.1 Å	5
	Maximum (Å)	0.34
Dihedral angle constraint violations	Number > 2.5 degrees	0
AMBER energies (kcal/mol)	Total	-1029.1
	Van der Waals	284.5
	Electrostatic	-1916.5