Supplementary Materials

The MC-SCV 4-step loop modeling protocol

The 4-step protocol for structure prediction was presented in reference (*1*). In the present application steps 2 – 4 are the same as previously described. However, in the present study the preparation of the variable segments (described in step 1) for the MC searches is different because here the MC-SCV is being used to analyze the crystal and mutant structures of the IL2 loop, whereas in (*1*) the MC-SCV is used to predict unknown loop structures.

Step-1, Generation of loop replicas. For *IL2* in β_1AR and β_2AR, the starting structure was based on the crystal structure coordinates (*2, 3*) and energy minimized before replication and carrying out Step-2, below. The β_1AR-*IL2* sequence is 3.55TSPF**R**YQSL**M**T3.65 with Arg at 3.59 and Met at 3.64 while the β_2AR-*IL2* sequence is 3.55TSPF**K**YQSL**L**T3.65 with Lys and Leu, at 3.59 and 3.64, respectively. Mutant *IL2* constructs were built using CHARMM (*4*) to introduce the mutation in the crystal structures of β_1AR, β_2AR and the newly modeled 5-HT_{2A}R. The initial structures of the mutants were based on the WT by copying coordinates of the common atoms into the mutant data set and calculating the coordinates of the remaining atoms using tools available in CHARMM (*4*).

For 5-HT_{2A}R, the initial *IL2* structure was built by attaching a previously modeled *IL2* (*5*) to the refined 5-HT_{2A}R homology model obtained from using rhodopsin and β_2AR as templates. TMH3 of the old and new models were aligned by using backbones of three sets of residues: nine residues: 3.47 to 3.55; seven residues: 3.49 to 3.55, and five residues: 3.51 to 3.55, respectively. This procedure yielded three differently positioned *IL2*. Due to the differences in the old and new 5-HT_{2A}R models, the C-terminus of the old *IL2* placed in the new model was not connected to TMH4. To close the loop, we replicated the three alignments 32 times each, and used the MC-SCV algorithm to close the open ends of the 96 structures to TMH4, which produced a total of 96 starting structures used in the subsequent steps as described.

Step-2, Open-Close Cycles at 310K (310-OCC). To relax the variable segments in the field of the protein and continuum solvent, a 310-OCC was carried out starting from the structures generated in Step-1. For β_1AR and β_2AR, crystal structures or mutants derived from crystal structures were replicated 128 times each for use in the 310-OCC. For the loop in WT 5-HT_{2A}R, 310-OCC was carried out for all the 96 starting structures obtained in Step-1. For mutant 5-HT_{2A}R, the structures obtained in Step-1 were replicated 128 times for use in the 310-OCC.

Step-3, High temperature OCC's. In earlier calculations it was found that conformations trapped in secondary minima of the energy landscape were unable to cross energy barriers and search the energy landscape for the native funnel(*6*). To overcome this problem (*1*) several (five in the present case) conformations were selected from the low energy region in Step-2, and each was replicated 128 times for a total of 640 conformations. The loops were then opened at 310 K with the harmonic constant *k* decreased from 1000 to 10^{-7} kcal/mol/Å^2 using a schedule $k_{i+1} = k_i/100$. When they were fully opened, they were heated to a high temperature (usually 1210 K or higher temperatures as indicated) in steps of 225 K with *k* kept at 10^{-7} kcal/mol/Å^2. Then the loops were closed, with *k* increased 10 fold at each step - from 10^{-7} to 100 kcal/mol/Å^2 at the high temperatures, to yield high temperature ("hot") conformations (HCs). Such open-close cycles are referred to as OHC-T where T is the final temperature of the HCs. Only one OHC was performed for each construct at temperatures higher than 1210 K, and for WT 5-HT_{2A}R, OHC-1210 was performed only on 384 conformations (4 ensembles each with 96 replicas).

Step-4, Identifying the native ensemble. In this step several low energy structures were selected from the Step-3 ensembles (OHC-T) and replicated, followed by 310-OCC to generate ensembles for which free energies were calculated. This step is used to test whether any of the low energy structures from Step-3 belong to the native ensemble, based on the rationale that if the structure belongs to the native ensemble, a 310-OCC will produce a compact ensemble with relatively small RMSD and energy spreads. This is so because the native funnel is surrounded

by high peaks and is thought to be a relatively narrow minimum so that the conformations of the ensemble generated at 310 K cannot escape (*1*). This hypothesis can be tested by carrying out an OCC starting from the crystal structure, which is assumed to belong to the native ensemble. To date, this test has worked for every case where it has been tried, including very long loops with up to ~20 residues.

Table S1. The conformations of the WT *IL2* in β_1AR after OHC at high temperatures and after 310-OCC.

Starting conf. from OHCs [a]				Results of 310-OCC [f]	
Temp (K) [b]	Conf and appearance [c]	Rank by energy [d]	Conf. [e]	Conf. distribution [g]	*ΔΔA* (kcal/mol) [h]
1210	25/11/13/13 [g]	1	Helix	**123**/5/0/0	**-18.48**
	(WT ensemble *1*	2	Helix	**120**/8/0/0	-17.46
	in Table 1;	3	Helix	**124**/3/1/0	-18.33
	Figure 2;	4	Helix	**116**/12/0/0	-17.74
	Figures 3A and	5	Helix	**115**/11/2/0	-16.29
	3B)	31	β_2-like	0/0/0/**128**	-14.35
		41	β_2-like	0/0/0/**93**	-0.28
		66	Partial-helix	0/**40**/0/15	11.00
		61	**Random**	2/14/**64**/0*	OCC1: -5.74
Additional 310-OCC		1	Helix	**123**/4/1/0*	OCC2: -12.29
		1	Helix	**122**/4/2/0	OCC3: -12.47
1435	0/1/1/16 [g]	1	**Helix-like**	**67**/7/54/0	-16.02
		2	Random	0/0/0/0	3.50
		3	**Random**	0/**1**/0/0	-0.24
		4	**Partial-helix**	1/**51**/14/32	0.40
		27	β_2-like	0/0/0/**123**	3.05
		5	**β_2-like**	10/9/22/**88***	OCC1: -4.23
Additional 310-OCC		1	Helix	**126**/2/0/0*	OCC2: -11.48
		1	Helix	**127**/1/0/0	OCC3: -11.63
1660	0/0/0/12 [g]	1	Random	0/0/0/**53**	-6.06
		3	**Helix-like**	**83**/9/36/0	-17.13

[a] Starting conformations for 310-OCC (Step-4) from OHC at [b] high temperatures (1210, 1435, 1660 and 2110 K).

[c] The conformation distribution of the hot ensemble from which replicas were taken and replicated for 310-OCC, and the corresponding figures and table where the ensemble were described.

[d] The energy ranking of the replica in its ensemble.

[e] The conformation of the "hot" replica as defined in Methods.

[f] The conformation distribution and energy of 310-OCC ensembles.

[g] The number of helices/partial helices/helix-like/β_2AR-like conformations in the 310-OCC ensemble as defined in Methods.

[h] The energy *ΔΔA* (in kcal/mol) of the 310-OCC ensemble was calculated as described in Methods.

* Additional 310-OCC was carried out using the replica from the current 310-OCC ensembles to explore conformational transitions at physiological temperature and the results were included in the next line. “Bold” denotes there is conformational transition in its 310-OCC, e.g., from random to helical or partial helical structures, or from partial-helix and helix-like to helical structures, and vice versa, the dominant conformation in the 310-OCC ensembles, or the 310-OCC ensemble with the lowest energy (*ΔΔA* or *ΔA*).

Table S2. The conformations of the WT *IL2* in β_2AR after OHC at high temperatures and after 310-OCC.

Starting conf. from OHCs [a]				Results of 310-OCC [f]	
Temp (K) [b]	Conf and appearance [c]	Rank by energy [d]	Conf. [e]	Conf. distribution [g]	*ΔΔA* (kcal/mol) [h]
1210	0/0/0/95 [g]	1	β_2-like	0/0/0/**128**	-19.12
		2	β_2-like	0/0/0/**128**	**-19.99**
		3	β_2-like	0/0/0/**128**	-19.73
		4	β_2-like	0/0/0/**128**	-14.94
		5	β_2-like	0/0/0/**128**	-19.77
1435	0/1/1/48 [g]	1	β_2-like	0/0/0/**128**	-19.42
	(Figures 3C	23	Helix-like	0/0/**128**/0	2.98
	an 3D)	22	**Partial-Helix**	0/2/**22**/5*	OCC1: -1.24
Additional 310-OCC		27	Partial-helix	0/**56**/71/0	OCC2: 2.65
1660	0/0/0/11 [g]	1	Random	0/0/0/0	1.57
		2	β_2-like	0/0/0/128	-10.24
		13	**Random**	0/2/1/**11**	OCC1: 13.69

Notes as in Table S1.

Table S3. The conformation distribution of the WT and reciprocal mutant *IL2* in β_1AR and β_2AR in OHC-1210 ensembles.

	Ensemble[a]	Helix[b]	Partial-helix[c]	helix-like[d]	β_2-like[e]	random coils[f]		Ensemble[a]	Helix[b]	Partial-helix[c]	helix-like[d]	β_2-like[e]	random coils[f]
A β_1AR	1	25	11	13	13	66	**B** β_1ILdm	1	16	12	9	14	77
	2	22	7	19	10	70		2	21	5	12	10	80
	3	31	9	9	11	68		3	25	14	10	9	70
	4	18	14	9	8	79		4	21	13	14	10	70
	5	31	13	10	8	66		5	23	9	9	12	75
	Total[g]	**127**	**54**	**60**	**50**	**349**		**Total**[g]	**106**	**53**	**54**	**55**	**372**
C β_2AR	1*	0(0)	0(1)	0(1)	95(48)	33(78)	**D** β_2ILdm	1	0	0	0	93	35
	2	0	0	0	103	25		2	0	0	1	82	46
	3	0	0	0	85	43		3	0	0	0	91	37
	4	0	0	0	91	37		4	0	0	0	88	40
	5	0	0	0	96	32		5	0	0	0	87	41
	Total[g]	**0**	**0**	**0**	**470**	**170**		**Total**[g]	**0**	**0**	**1**	**441**	**199**

Notes as in Table 2.

* The conformation distribution of an OHC-1435 ensemble is shown in parenthesis.

Table S4. The conformations of β_1IL2dm after OHC-1210 and after 310-OCC.

Starting conf. from OHCs [a]				Results of 310-OCC [f]	
Temp (K) [b]	Conf and appearance [c]	Rank by energy [d]	Conf. [e]	Conf. distribution [g]	*ΔA* (kcal/mol) [h]
1210	16/12/9/14 [g] (Figures 5A and 5B)	1	**Partial-helix**	6/30/**49**/0	-5990.56
		2	Helix	**126**/2/0/0	-5990.27
		3	**Partial-helix**	**104**/8/16/0	-5989.29
		4	Helix	**127**/1/0/0	**-5990.59**
		5	Helix	**125**/3/0/0	-5987.98
		34	β_2-like	0/0/0/**120**	*-5977.81*
		46	β_2-like	0/0/0/**129**	-5980.63
Additional 310-OCC		41	**Random**	1/**75**/50/0*	OCC1: -5983.06
		1	**Partial-helix**	7/20/**64**/0*	OCC2: -5985.22
		1	Helix	3/**24**/101/0	OCC3: -5986.05

Notes as in Table S1.

Table S5. The conformations of β_2IL2dm after OHC-1210 and -1435, and after 310-OCC.

Starting conf. from OHCs [a]				Results of 310-OCC [f]	
Temp (K) [b]	Conf and appearance [c]	Rank by energy [d]	Conf. [e]	Conf. distribution [g]	*ΔA* (kcal/mol) [h]
1210	0/0/0/95 [h]	1	β_2-like	0/0/0/**128**	-7347.44
	(Figures 5C	2	β_2-like	0/0/0/**128**	-7345.74
	and 5D)	3	β_2-like	0/0//0/**128**	**-7348.20**
		4	β_2-like	0/0/0/**128**	-7338.76
		5	β_2-like	0/0/0/**128**	-7346.75
		78	**Random**	0/0/0/**8***	OCC1: -7327.26
Additional 310-OCC		7	β_2-like	0/0/0/**128**	OCC2: -7344.04
1210	0/0/0/95 [h] (Fig. 4C-D)	87	Helix-like	0/0/**96**/0*	OCC1: -7310.35
Additional 310-OCC		47	helix-like	0/0/**104**/0	OCC2: -7311.74
1435	0/1/1/48 [h]	1	β_2-like	0/0/0/**128**	-7338
		19	Helix-like	0/0/**127**/0	-7314

Notes as in Table S1.

Table S6. The conformation distribution of OHC-1210 ensembles of the WT and P3.57A mutant *IL2* in 5-HT_{2A}R.

	Ensemble [a]	helix [b]	Partial-helix [c]	helix-like [d]	β_2-like [e]	random coil [f]
A	1	0	1	7	14	74
WT	2	0	0	3	23	70
	3	1	0	3	19	73
	4	0	0	3	17	76
Total [g]	**384**	**1**	**1**	**16**	**73**	**293**
B	1	2	0	6	6	114
	2	0	2	0	13	113
P3.57A	3	1	1	2	16	108
	4	2	1	2	21	102
	5	0	1	2	17	108
Total [g]	**640**	**5**	**5**	**12**	**73**	**545**

Notes as in Table 1.

Table S7. The conformations of the WT and P3.57A mutant *IL2* in 5-HT_{2A}R after OHC-1210 and after 310-OCC.

	Starting Conf. [a]		Results of 310-OCC [f]	
	Conf. [d]	Energy Ranking [e]	Conf. distribution [g]	*ΔA* (kcal/mol) [h]
WT	Helix	1	**123**/4/1/0	**-4503.30**
	Random	3	0/0/0/0	-4502.17
	Helix-like	2	0/0/**128**/0	-4497.21
	Random	4	0/0/0/0	-4496.29
	Helix-like	5	0/8/**118**/0	-4495.79
P3.57A	Random	4	0/**3**/0/0	**-4352.81**
	Helix	3	1/**10**/0/3	-4348.19
	Random	1	0/**7**/0/2	-4347.80
	Helix	2	0/**6**/2/2	-4344.23
	Random	5	0/**3**/0/1	-4338.63

Notes as in Table 2.

References

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