Electronic Supplementary Material (ESI) for Molecular BioSystems.

Electronic Supplemental Information

Structural Complexes of Agonist, Inverse Agonist and Antagonist bound C5a Receptor: Insights into Pharmacology and Signaling†

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Supplemental Figures S1 to S10 and Table S1 are provided.

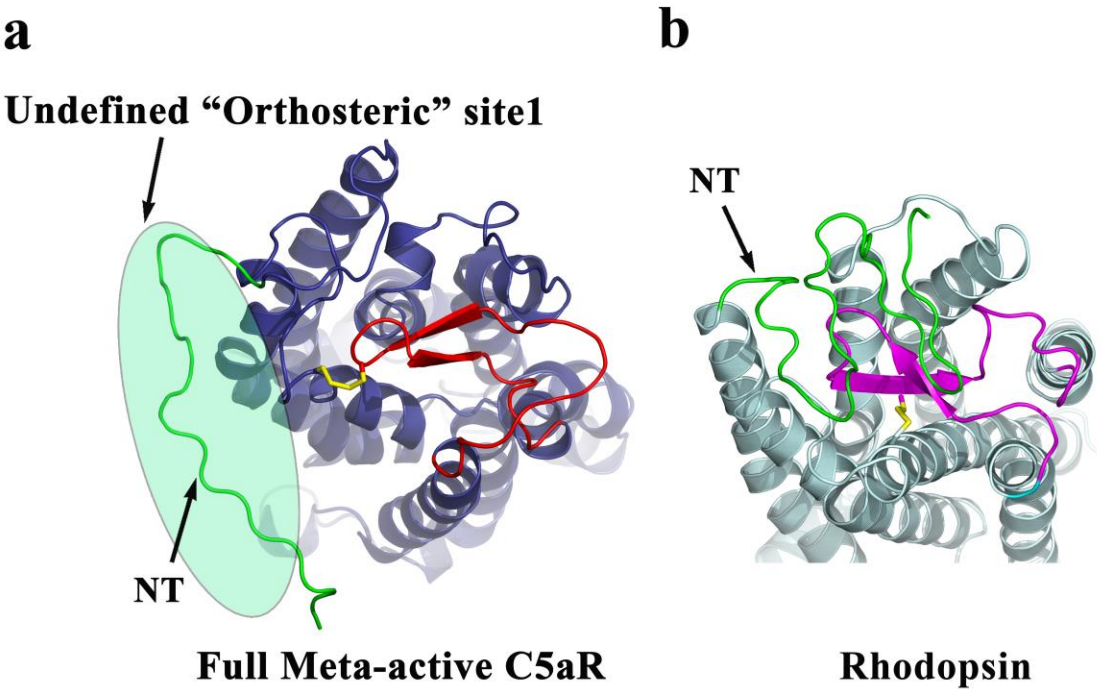

Figure S1. Comparison of the orientation of N-terminus between the meta-active C5aR and Rhodopsin.

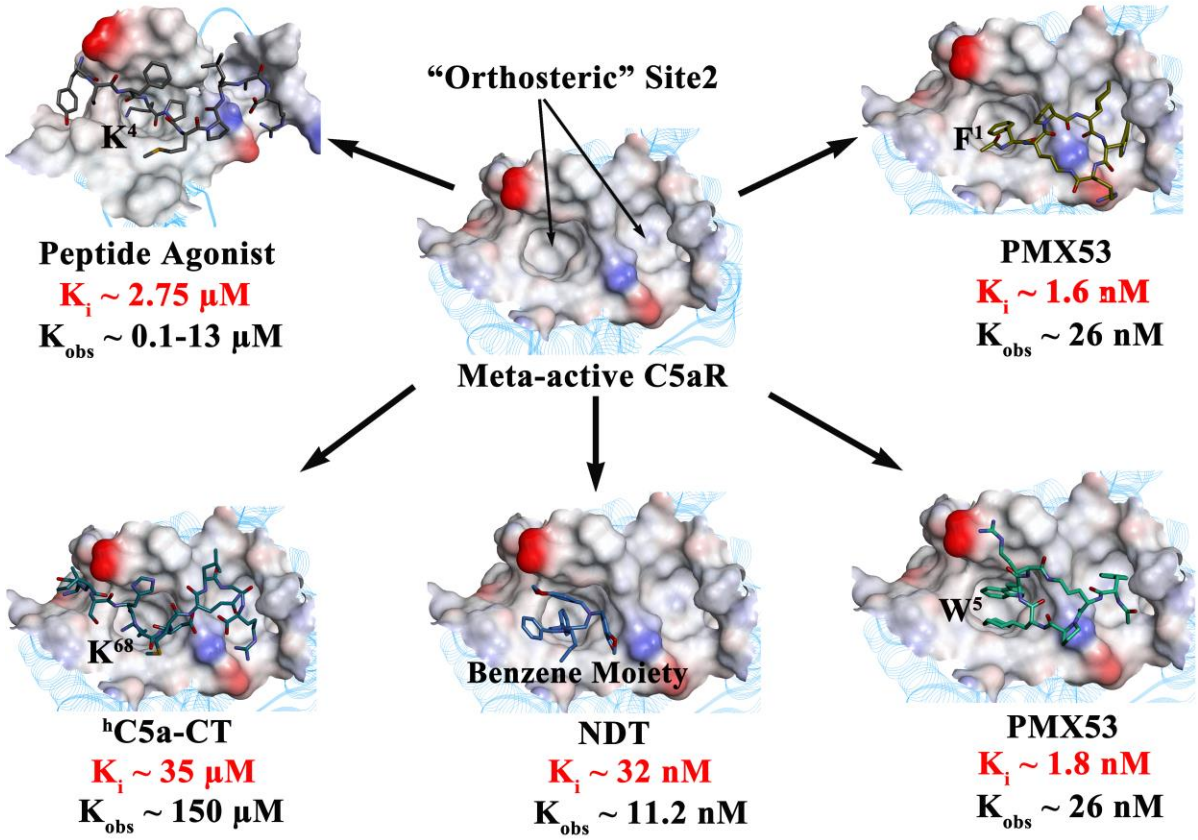

Figure S2. Summary of the binding modes respectively illustrated by the engineered peptide agonist, native agonist ^{h}C5a-CT, inverse agonist NDT 9513727 and cyclic peptide antagonist PMX53 at the "orthosteric" site2 of the full meta-active C5aR. K_i and K_{obs} respectively represents the estimated affinity and the experimentally observed affinity for the ligands.

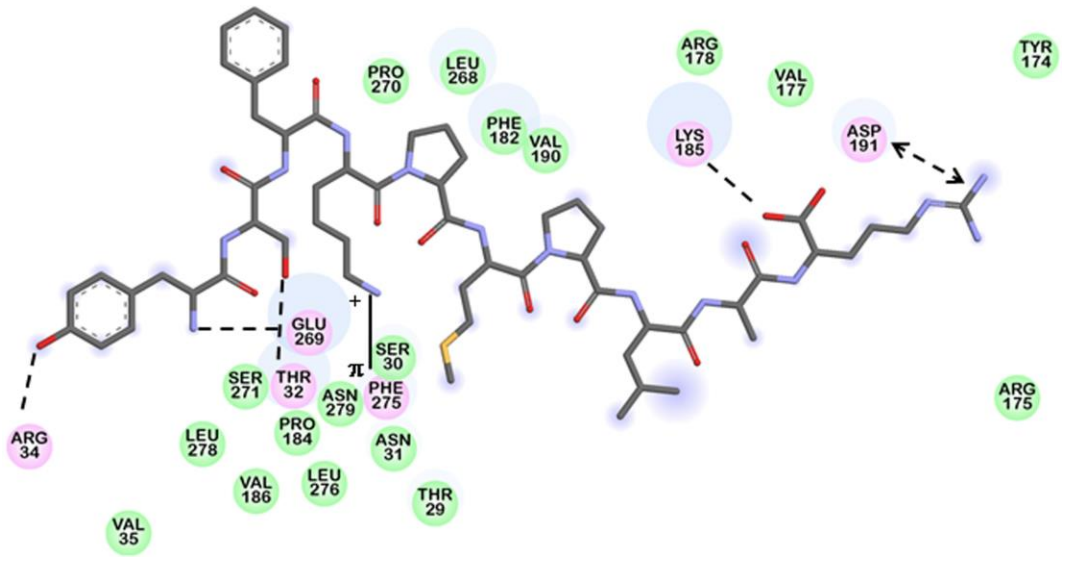

Figure S3. 2D interaction plot of the engineered peptide agonist demonstrating the intermolecular interaction specificity at the "orthosteric" site2 of the meta-active C5aR, where the K^4 occupies the major groove at the "orthosteric" site2 of C5aR. Green and pink circles respectively represent the hydrophobic and polar residues contributing toward the interactions at the "orthosteric" site2 of C5aR. H-bonds are represented as black dashed lines. Cation- π and Salt-bridge interactions are shown respectively as black solid lines and black dotted arrows.

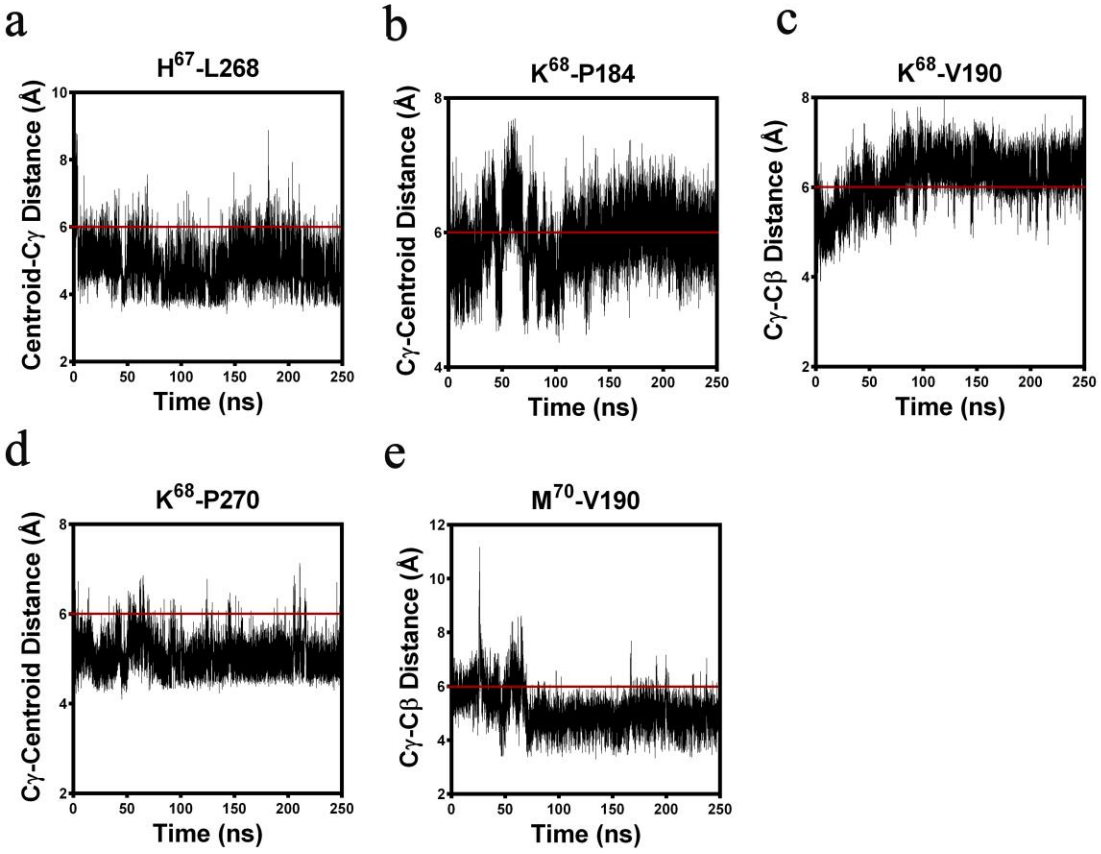

Figure S4. Summary of the hydrophobic interactions observed between the ^{h}C5a-CT and the meta-active C5aR, over 250 ns of MD at 300K in POPC bilayer. H^{67}, K^{68}, and M^{70}, respectively represent the interacting residues on the ^{h}C5a-CT. L268, P184, V190 and P270 respectively represent the interacting residues on the C5aR. (a) Strong interaction between the centroid of imidazole ring of H^{67} with Cγ of L268. (b) The strong hydrophobic interaction between Cγ of K^{68} and centroid of P184. (c) Moderate interaction between the Cγ of K^{68} and Cβ of V190. (d) Stable

hydrophobic interaction between the Cγ of K^{68} and centroid of P270. (e) Strong hydrophobic interaction between Cγ of M^{70} and Cβ of V190.

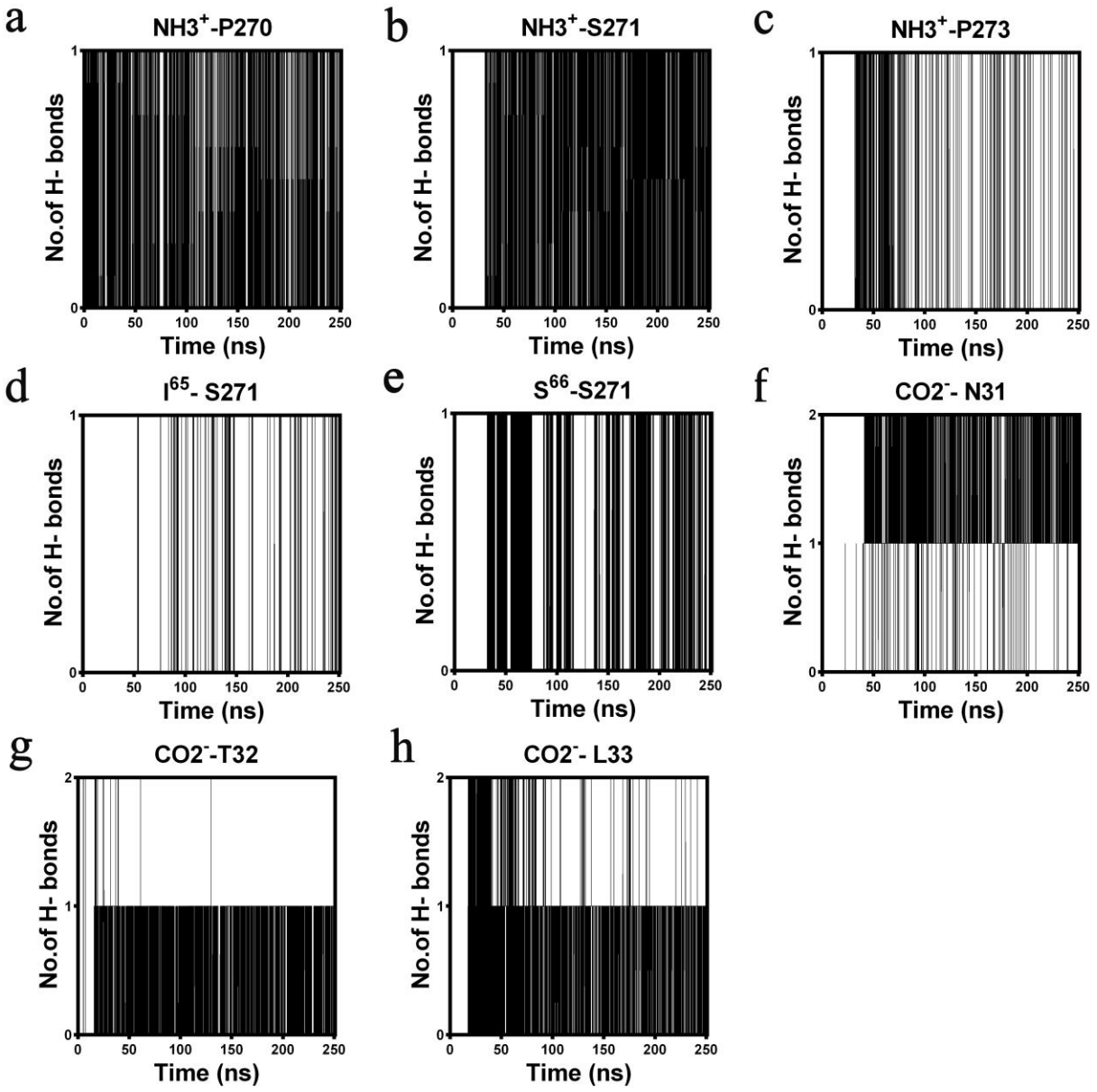

Figure S5. Summary of the intermolecular hydrogen bond interactions observed between the ^{h}C5a-CT and the meta-active C5aR, over 250 ns of MD at 300K in POPC bilayer. $NH3^{+}$, CO_2^{-}, I^{65}, and S^{66}, respectively represent the interacting residues on the ^{h}C5a-CT. N31, T32, L33, P270, S271, and P273, respectively represent the interacting residues on the C5aR. (a) Strong hydrogen bonding between $NH3^{+}$ of N^{64} and backbone carbonyl group of P270. (b) Strong hydrogen bonding between $NH3^{+}$ of N^{64} and backbone carbonyl group of S271. (c) Strong hydrogen bonding between $NH3^{+}$ of N^{64} and backbone carbonyl group of P273. (d) Moderate hydrogen bonding between backbone

carbonyl of I^{65} and sidechain hydroxyl group of S271. (e) Strong hydrogen bonding between backbone NH of S^{66} and sidechain hydroxyl group of S271. (f) Strong hydrogen bonding between CO_2^- of R^{74} and sidechain NH2 of N31. (g) Strong hydrogen bonding between CO_2^- of R^{74} and sidechain hydroxyl group of T32. (h) Strong hydrogen bonding between CO_2^- of R^{74} and backbone NH of L33.

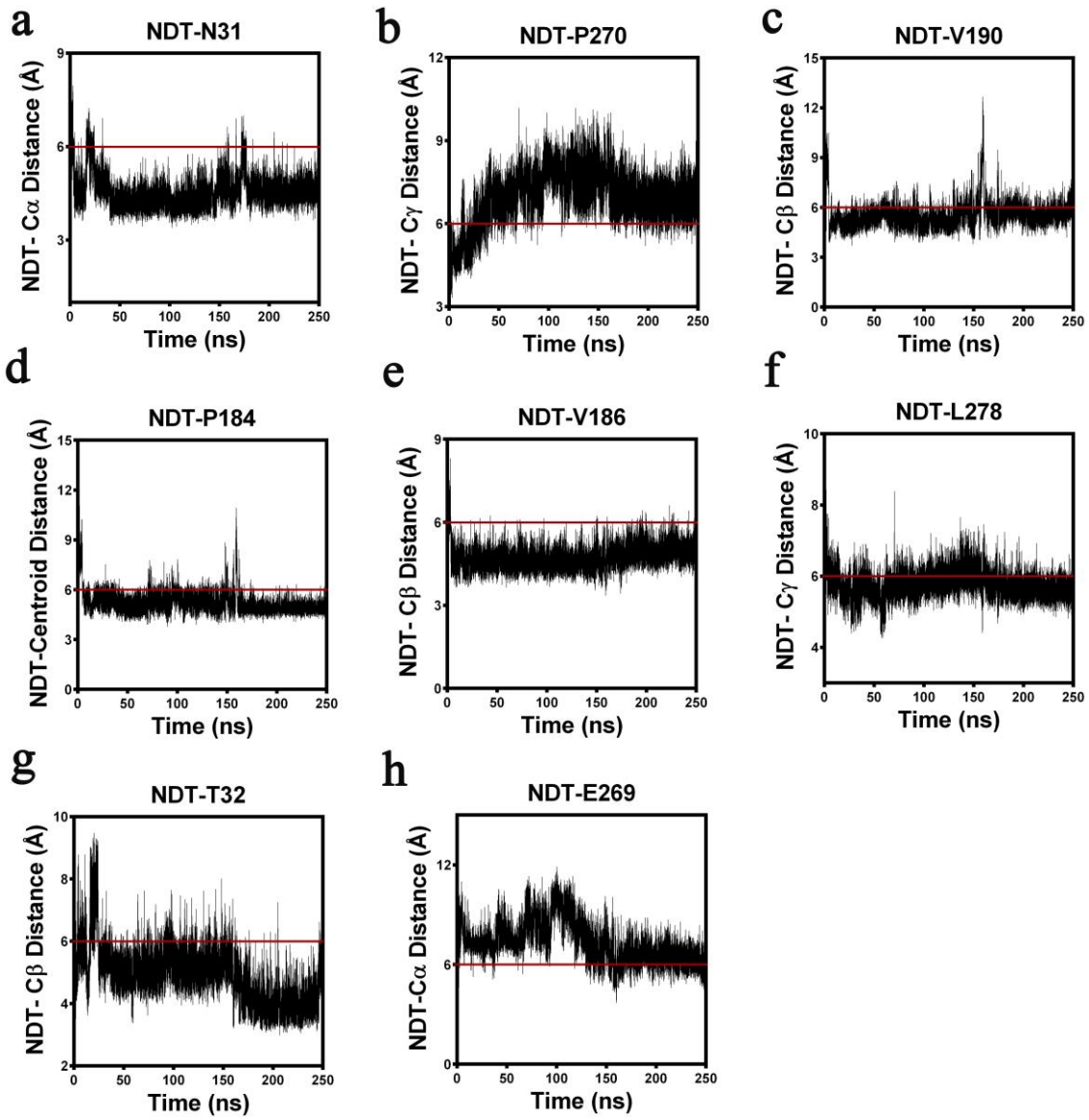

Figure S6. Summary of hydrophobic interactions observed between the NDT and meta-active C5aR, over 250 ns of MD at 300K in POPC bilayer. N31, T32, L278, P184, V186, V190, E269 and P270 respectively represent the interacting residues on the C5aR. (a) Presence of strong interaction between the centroid of 5-membered ring of NDT with Cα of N31. (b) Moderate hydrophobic

interaction between centroid of 5-membered ring of NDT and Cγ of P270. Moderate hydrophobic interaction between the centroid of 6-membered ring of benzodioxol moiety of NDT with Cβ of V190 (c) and with centroid of 5-membered ring of P184 (d). (e) Stable hydrophobic interaction between the centroid of the benzene moiety of NDT and Cβ of V186. (f) Presence of hydrophobic interaction between the centroid of the benzene moiety of NDT and Cγ of L278. (g) Stable hydrophobic interaction between the centroid of the other benzene moiety of NDT and Cβ of T32. (h) Moderate hydrophobic interaction between the centroid of 6-membered ring of benzodioxol moiety of NDT with Cα of E269.

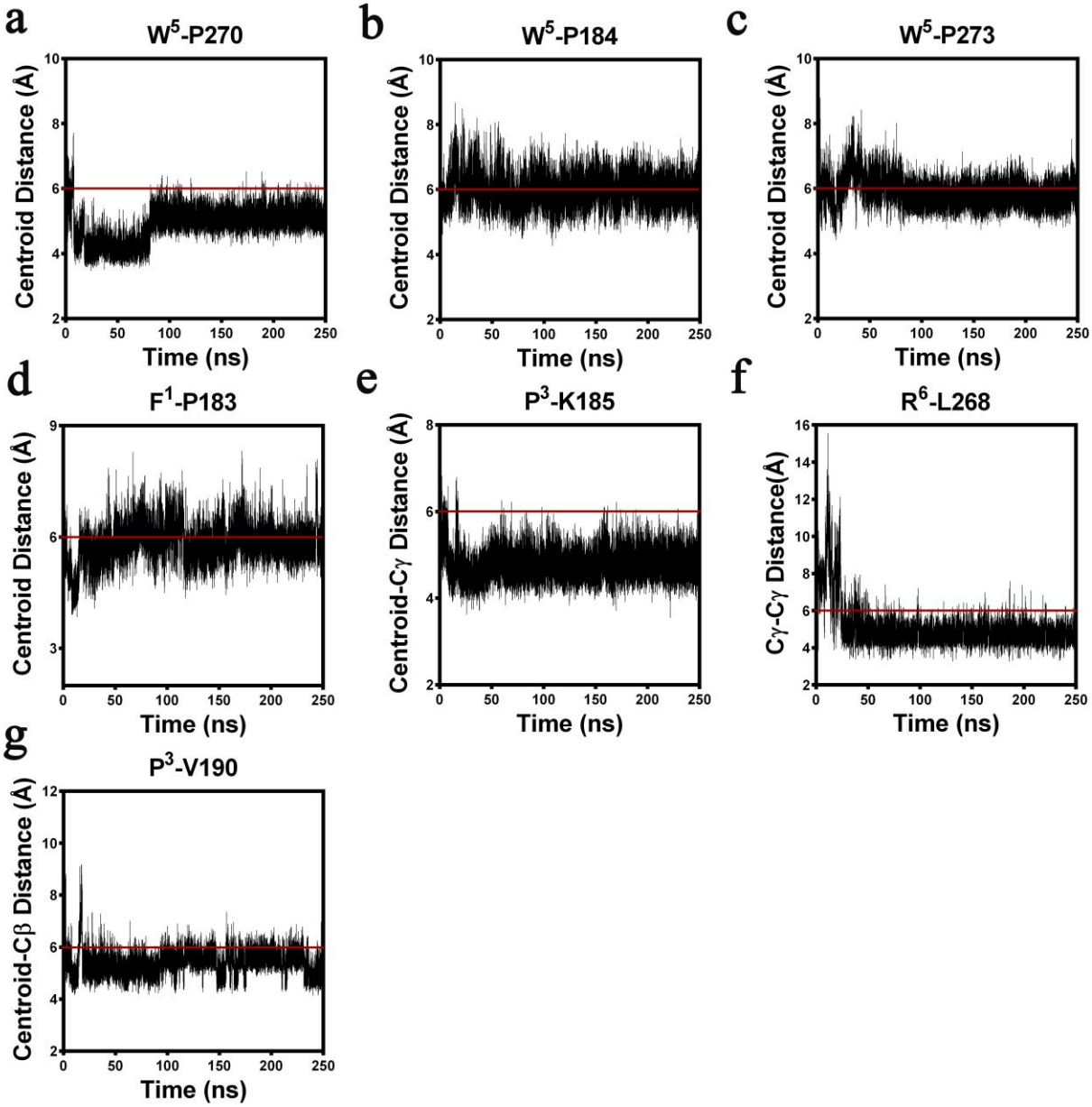

Figure S7. Monitoring the stability of the hydrophobic interactions over 250 ns of MD at 300K in POPC bilayer, observed between PMX53 and meta-active C5aR. Stable hydrophobic interactions observed between the centroids of the 5-membered ring of W^5 with P270 (a), with P184 (b) and with P273(c). (d) Presence of strong interaction between the centroids of F^1 and P183. (e) The strong hydrophobic interaction between centroid of P^3 and Cγ of K185. (f) Presence of strong interaction between Cγ atoms of R^6 and L268. (g) Strong hydrophobic interaction between centroid of P^3 and Cβ of V190. The superscripts indicate that the residues are part of the PMX53.

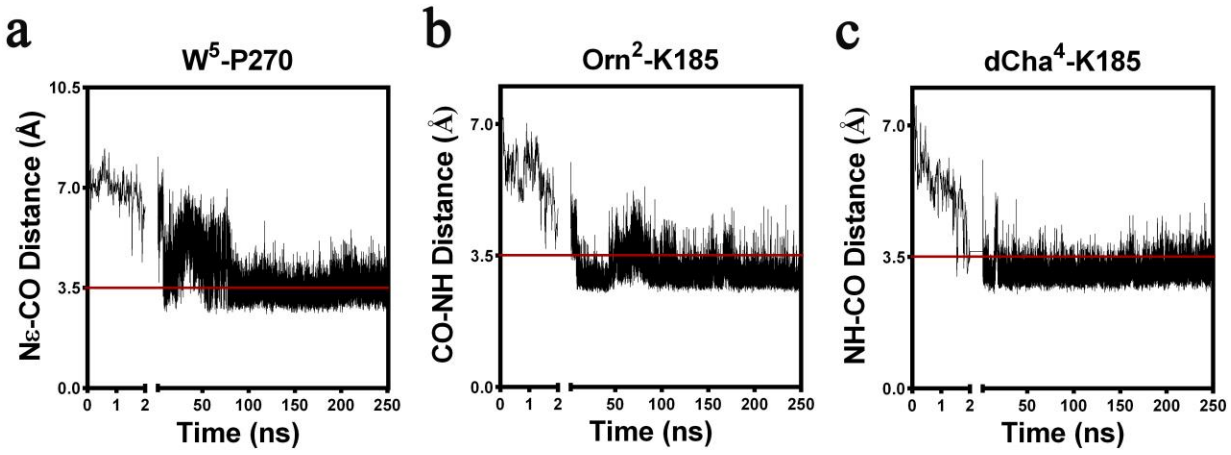

Figure S8. Summary of hydrogen bonding interactions observed between the PMX53 and meta-active C5aR, over 250 ns of MD at 300K in POPC bilayer. (a) Strong hydrogen bonding interaction between indole nitrogen on W^5 with backbone carbonyl of P270. (b) Stable hydrogen bonding interactions observed between the backbone carbonyl of Orn2 and backbone NH of K185. (c) Presence of strong hydrogen bonding interactions observed between the backbone NH of $dCha^4$ and carbonyl of K185.

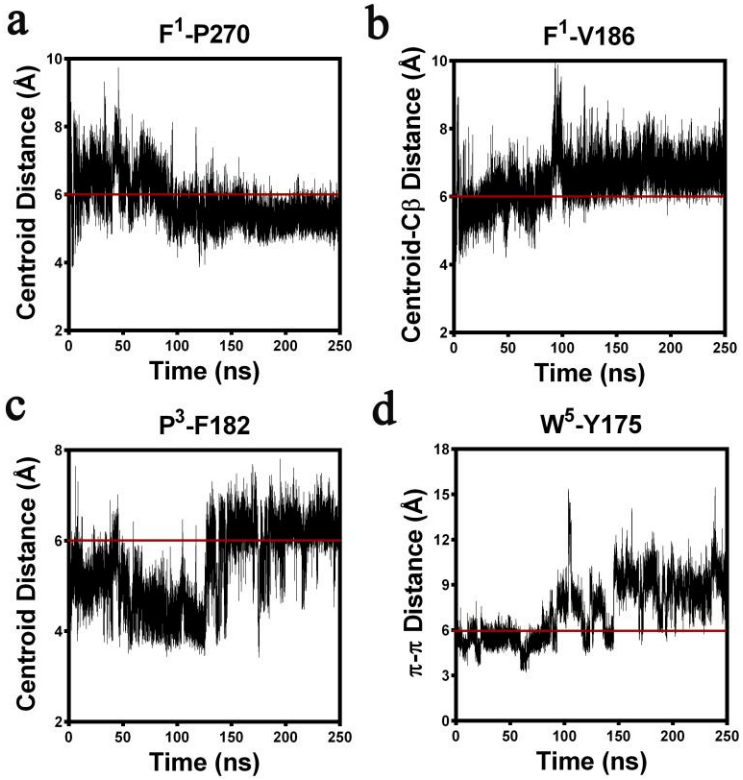

Figure S9. Summary of hydrophobic caging interactions observed for the other binding mode of PMX53 with meta-active C5aR, over 250 ns of MD at 300K in POPC bilayer. (a) Stable hydrophobic interactions observed between the centroids of F^1 and P270. (b) Moderate hydrophobic interaction between centroid of F^1 and Cβ of V186. (c) Presence of Moderate hydrophobic interactions observed between the centroids of P^3 and F182. (d) Weaker π-π interaction noted between W^5 and Y175. The superscripts indicate that the residues are part of the PMX53.

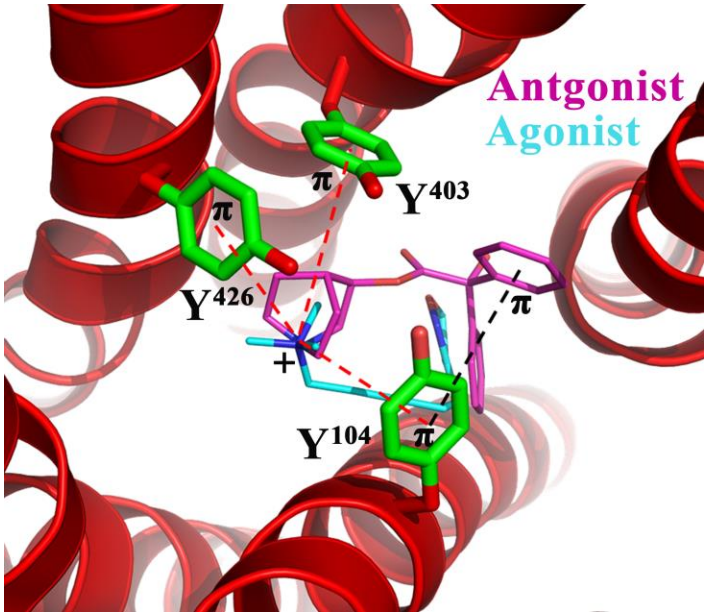

Figure S10. Comparison of the binding interactions observed between the agonist (PDB: 4MQS) and antagonist (PDB: 3UON) bound M2R. Y104, Y403 and Y426 represent the residues of M2R. Agonist (cyan) and antagonist (pink) respectively demonstrate "cation-π" and "π-π" interactions with the residues of M2R. "cation-π" and "π-π" interactions respectively represented as red and black dashed lines.

Table S1. Details of the "cation-π" and "π-π" type interactions observed in the known structural complexes of GPCRs with variety of ligands.

PDB ID	Ligand	Interacting residues (Receptor)	π-π Interaction Types	Distance (Å)	Ligand Type
3REY (A2AR)	XAC999	F168	Stacked	3.7	Antagonist
			Stacked	4.56	
4EJ4 (δ- Opioid)	EJ4500	W284	T-shaped	5.33	Antagonist
4MBS (CCR5)	MRV	F112	T-shaped	5.04	Inverse agonist
		Y108	T-shaped	5.15	
		F109	T-shaped	5.13	
		Y251	Stacked	5.47	
		W86	Stacked	4.41	
			Stacked	5.01	
3RZE (Histamine)	Doxepin	F432	T-shaped	4.8	Antagonist
		F435	T-shaped	5.6	
		Y108	T-shaped	4.7	
		W428	T-shaped	4.6	
			T-shaped	4.9	
2RH1 (β2AR)	Carazol	F290	T-shaped	4.68	Inverse agonist
			T-shaped	5.2	
		F193	T-shaped	5.06	
		Y199	T-shaped	5.89	
			T-shaped	5.98	
4DAJ (M3R)	Tiotropium	Y148	T-shaped	5.5	Inverse agonist
		W199	Stacked	4.72	
3VW7 (PAR1)	Vorapaxar	F271	Stacked	5.5	Antagonist
		Y183	T-shaped	4.7	
3PBL (D3R)	Eticlopride	F345	T-shaped	5.3	Antagonist
3UON (M2R)	3-Quinuclidinyl-benzilate	Y104	T-shaped	5.64	Antagonist
3P0G (β2AR)	BI-167107	F290	T-shaped	4.9	Agonist

3QAK (A2AR)	UK-432097	Y271	T-shaped	5.6	Agonist
		F168	Stacked	3.6	
			Stacked	3.5	
4MQS (M2R)	Iperoxo		Cation-π (3)		Agonist
4PXZ (P2Y12)	2MeSADP	Y105	Stacked	4.49	Agonist
			Stacked	3.89	
4GRV (NT-R1)	Neurotensin	F344: R8	Cation-π (2)	4.3	Agonist
		F311: R9		4.43	
			Salt-bridge (1)		
4DJH (κ-Opioid)	JDTiC	W124	Stacked	5.25	Antagonist
4XNV (P2Y1)	BPTU	F62	T-shaped	4.99	Antagonist
		F119	T-shaped	5.04	
4YAY (AT1R)	ZD7155	W84	Stacked	4.48	Antagonist
			Stacked	4.4	
4NTJ (P2Y12)	AZD1283	F252	T-shaped	5.4	Antagonist
		Y105	Stacked	4.06	
2Y00 (β1AR- Turkey)	Dobutamine	F307	T-shaped	5.19	Partial agonist
2YCY (β1AR)	Cyanopindolol	F307	T-shaped	4.73	Antagonist
4UG2 (A2AR)	CGS21680	F168	Stacked	3.76	Agonist
			Stacked	3.74	
4RWD (δ-Opioid)	Tetra-peptide	W284: Tic2	T-shaped	5.25	Antagonist
		Y129: H-Dmt	T-shaped	5.6	
4XT1 (US28-CX3CL1)	CX3CL1	Y92: K7	Cation-π	4.5	Agonist
		F111: H2	Stacked	4.07	
		W89: H2	T-shaped	5.08	
			T-shaped	4.7	