

Extracellular domain stability determines VIP affinity in VPAC1, VPAC2, and PAC1 Variants

Supplementary Material

Supplementary Table T1: Models stereochemical quality validation

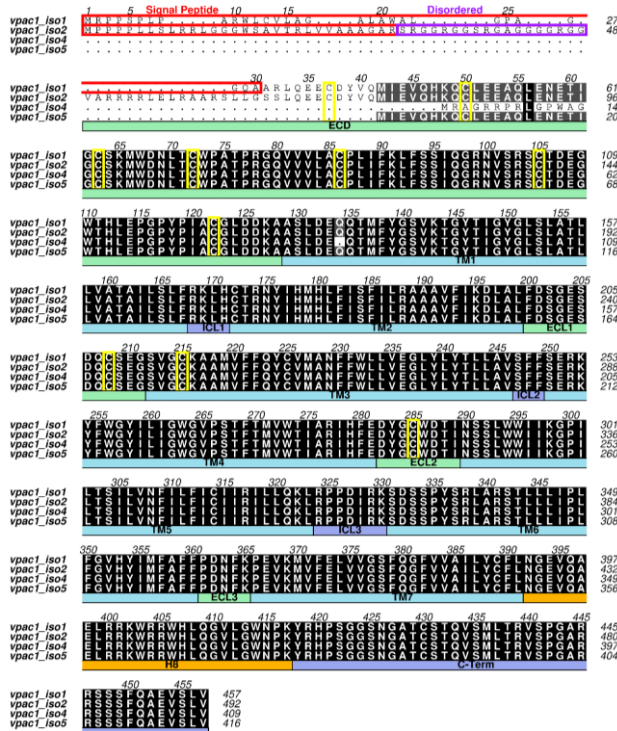
	MolProbity Score	Outliers rotamers	Ramachandran Favorable	Ramachandran Outliers	QMEANDisCo	RMSD (Å)
Reference Values	≈ 0	< 1%	> 98%	< 0,2%	≈ 1,00	≈ 0
PAC1 (PDB ID 6LPB)	1,68	1,56	95,34	0,00	0,74	-/-
VPAC1 (PDB ID 8E3Z)	1,62	0,32	96,21	0,00	0,77	-/-
VPAC2 (PDB ID 7VQX)	1,65	0,00	95,81	0,00	0,78	-/-
VPAC1-iso1	2,24	6,08	91,48	1,66	0,64	4,51
VPAC1-iso2	2,70	12,14	89,92	2,52	0,58	6,62
VPAC1-iso3	2,93	10,54	85,98	4,06	0,47	9,46
VPAC1-iso4	1,94	3,71	91,92	1,62	0,64	3,99
VPAC1-iso5	2,03	5,05	92,05	1,59	0,63	3,78
VPAC2-iso1	2,00	3,49	92,64	1,52	0,61	3,26
VPAC2-iso2	2,70	7,02	81,50	5,75	0,56	9,32
VPAC2-iso2 (Modeller)	2,00	3,23	95,54	0,84	0,54	6,86
PAC1-iso1	2,78	13,69	89,63	2,03	0,58	3,38
PAC1-iso2	2,36	6,00	91,35	2,69	0,55	3,93
PAC1-iso3	2,34	5,85	92,18	1,20	0,61	3,29
PAC1-iso4	2,38	6,10	91,78	1,40	0,59	3,82
PAC1-iso5	1,99	3,83	92,18	1,38	0,60	8,14

The RMSD was calculated against the PBDs entry for each receptor.

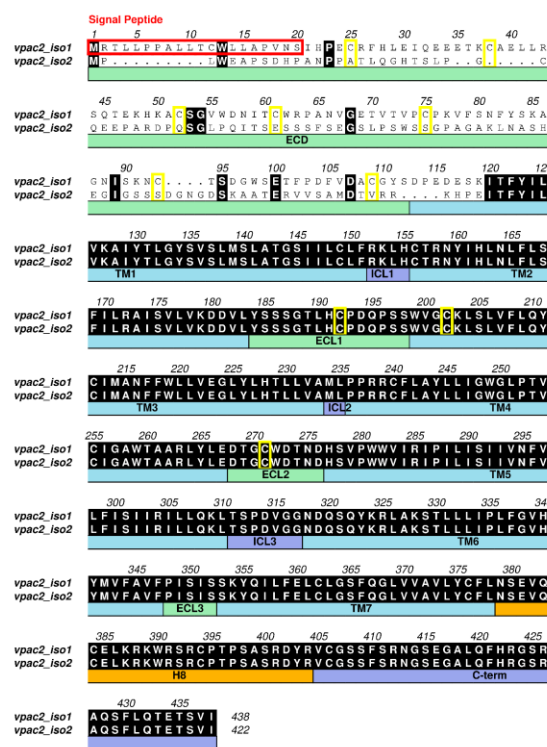
Supplementary Table T2: MM/GBSA binding energy estimative in kcal/mol.

Systems	ΔE_{vdw}	ΔE_{Elec}	ΔE_{GB}	ΔE_{SURF}	ΔG_{GAS}	ΔG_{SOLV}	ΔG_{TOTAL}
VPAC1-iso1	-189 \pm 14	-856 \pm 70	956 \pm 66	-28 \pm 2	-1044 \pm 76	927 \pm 65	-117 \pm 17
VPAC1-iso2	-168 \pm 9	-519 \pm 88	630 \pm 78	-24 \pm 1	687 \pm 93	605 \pm 77	-82 \pm 19
VPAC1-iso4	-168 \pm 15	-350 \pm 106	459 \pm 103	-24 \pm 2	-518 \pm 105	435 \pm 102	-83 \pm 12
VPAC1-iso5	-158 \pm 12	-590 \pm 99	675 \pm 93	-24 \pm 1	-748 \pm 101	651 \pm 92	-97 \pm 13
VPAC2-iso1	-151 \pm 10	-434 \pm 90	517 \pm 85	-23 \pm 2	-585 \pm 95	494 \pm 84	-91 \pm 16
VPAC2-iso2	-167 \pm 14	-195 \pm 95	296 \pm 95	-25 \pm 2	-362 \pm 97	271 \pm 94	-90 \pm 12
PAC1-iso1	-165 \pm 9	-770 \pm 58	878 \pm 57	-25 \pm 1	-935 \pm 58	853 \pm 57	-82 \pm 10
PAC1-iso2	-168 \pm 11	-997 \pm 100	1084 \pm 91	-26 \pm 2	-1165 \pm 104	1058 \pm 91	-107 \pm 26
PAC1-iso3	-167 \pm 10	-769 \pm 70	855 \pm 63	-25 \pm 1	-935 \pm 68	829 \pm 63	-106 \pm 15
PAC1-iso4	-160 \pm 20	-780 \pm 93	878 \pm 101	-23 \pm 3	-940 \pm 107	854 \pm 98	-86 \pm 15
PAC1-iso5	-182 \pm 15	-858 \pm 81	943 \pm 79	-27 \pm 2	-1040 \pm 88	916 \pm 78	-124 \pm 16

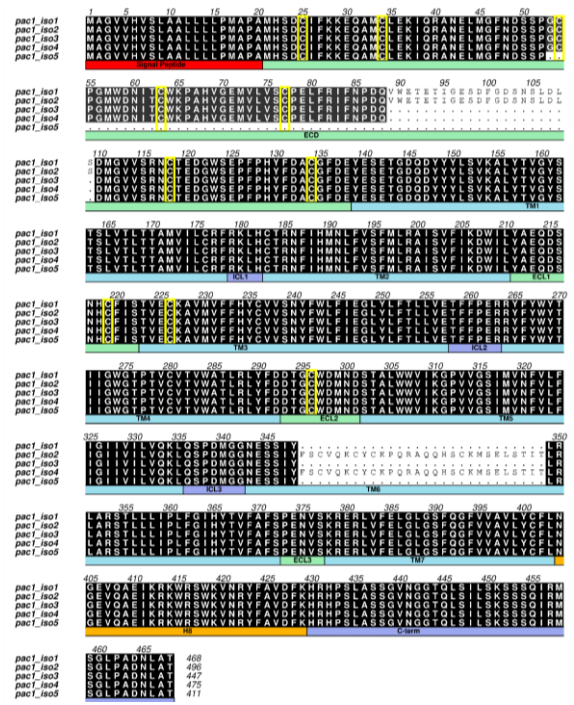
A)



B)



C)



Supplementary Figure S1: Multiple alignments of receptor isoforms. A) VPAC1. B) VPAC2. C) PAC1. Residues are colored according to their similarity, with conserved residues colored black and non-conserved residues uncolored. Conserved GPCR domains are identified according to their position relative to the membrane, in the boxes below the sequences: extracellular (green), transmembrane (cyan), intracellular (purple), intracellular α-helix parallel to the membrane (orange). The squares around the residues indicate: signal peptide (red), disordered region (purple), cysteines that form disulfide bonds (yellow).