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Supplementary information

Activation of the GLP-1 receptor by a non-peptidic agonist

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Supplemental Table 1. CryoEM data collection, refinement and model parameters

	TT-OAD2:GLP1R: Gs (EMDB-20179) (PDB 6ORV)
Data collection and processing	
Magnification	105,000 (indicated); 60,530 (actual)
Voltage (kV)	300
Electron exposure (e–/Å^2)	65.6 and 63.5 (super resolution)
Defocus range (μm)	0.7 – 1.5
Pixel size (Å)	0.826
Symmetry imposed	C1
Initial particle images (no.)	1,470,000
Final particle images (no.)	85,978
Map resolution (Å)	3.0
FSC threshold	0.143
Map resolution range (Å)	200-1.65
Refinement	
Initial model used (PDB code)	6B3J
Model resolution (Å)	3.0
FSC threshold	0.143
Model resolution range (Å)	999-1.65
Map sharpening *B* factor (Å^2)	-70
Model composition	
Non-hydrogen atoms	8041
Protein residues	997
Ligands	1
B factors (Å^2)	
Protein	38
Ligand	76
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	0.857
Validation	
MolProbity score	1.56
Clashscore	4.89
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	95.7
Allowed (%)	4.3
Disallowed (%)	0

Supplemental Table 2. Effect of GLP-1R mutations on cell surface expression and cAMP signalling. cAMP data in figure 2 were analysed using a three-parameter logistic equation to derive pEC_{50} and Emax values. pEC_{50} values represent the negative logarithm of the concentration of agonist that produces half the maximal response. Emax are presented as a % of the WT response. The same data was analysed using the operational model of agonism to derive functional affinity values (Log K_A) and efficacy values (Logτ) that were corrected to the cell surface expression data as determined by ELISA. These are reported as Logτc. All values are expressed as mean ± S.E.M. of four individual experiments.

Construct	Cell surface Expression (% WT)	pEC50	Emax (% WT)	LogK_A	Logτ_c
Wildtype	-	6.88 ± 0.09	100 ± 2	-6.55 ± 0.12	0.195 ± 0.05
$Y145^{1.40}A$	88 ± 8	6.09 ± 0.34	55 ± 9	-6.01 ± 0.25	-0.300 ± 0.16
$Y148^{1.43}A$	67 ± 8	ND	ND	ND	ND
$K197^{2.67}A$	22 ± 4	ND	ND	ND	ND
$L201^{2.71}A$	94 ± 2	5.73 ± 0.36	51 ± 9	-5.55 ± 0.33	-0.346 ± 0.16
$W203^{2.73}A$	94 ± 6	6.53 ± 0.27	50 ± 4	-6.30 ± 0.31	-0.363 ± 0.09
$M204^{2.74}A$	99 ± 2	5.86 ± 0.45	42 ± 11	-5.70 ± 0.34	-0.488 ± 0.15
$L217^{ECL1}A$	109 ± 5	6.55 ± 0.34	69 ± 7	-6.31 ± 0.46	-0.212 ± 0.09
$Y220^{ECL1}A$	94 ± 4	5.07 ± 0.82	14 ± 9	ND	ND
$F230^{3.33}A$	92 ± 6	6.26 ± 0.34	55 ± 8	-6.03 ± 0.29	-0.292 ± 0.11
$M233^{3.36}A$	126 ± 7	5.80 ± 0.30	13 ± 2	-5.77 ± 0.53	-1.22 ± 0.42
$T298^{ECL2}A$	85 ± 6	6.65 ± 0.40	64 ± 7	-6.41 ± 0.36	-0.152 ± 0.13
$W297^{ECL2}A$	60 ± 7	5.85 ± 0.21	44 ± 5	-5.69 ± 0.40	-0.314 ± 0.17

Supplemental Table 3. GLP-1R systems prepared and simulated in the present work. Besides the GLP-1R:Gs protein complexes bound to TT-OAD2 (GLP-1R:TT-OAD2:Gs) and GLP-1 (GLP-1R:GLP-1:Gs), the two corresponding complex without Gs protein (GLP-1R:TT-OAD2 and GLP-1R:GLP-1, respectively) and the two apo receptors (apo-GLP-1R (TT-OAD2) and apo-GLP-1R (GLP-1), respectively) were prepared as described above and subject to four MD replicas each.

GLP-1R System	Number of replicas	Total MD simulation time
GLP-1R:TT-OAD2:Gs	4	2 μs
GLP-1R:TT-OAD2	4	4 μs
Apo-GLP-1R (TT-OAD2)	4	4 μs
GLP-1R:GLP-1:Gs	4	2 μs
GLP-1R:GLP-1	4	4 μs
Apo-GLP-1R (GLP-1)	4	4 μs