

Supplemental Information

Ligand recognition and allosteric regulation of DRD1-Gs signaling complexes

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Table S1. Cryo-EM data collection, refinement and validation statistics. Related to Figure 1.

Data collection and processing	Fenoldopam-DRD1-Gs-Nb35	A77636-DRD1-Gs-Nb35	SKF83959-DRD1-Gs-Nb35	PW0464-DRD1-Gs-Nb35	Dopamine-PAM-DRD1-Gs-Nb35
Magnification	22,500	22,500	105,000	22,500	22,500
Voltage (kV)	300	300	300	300	300
Electron exposure (e ⁻ /Å)	64	64	44	64	64
Defocus range (μm)	-1.0~-2.0	-1.0~-2.0	-1.0~-2.5	-1.0~-2.0	-1.0~-2.0
Pixel size (Å)	1.0	1.0	0.85	1.0	1.0
Symmetry imposed	C1	C1	C1	C1	C1
Initial particle images (no.)	2,386,124	1,593,784	1,545,798	2,361,261	2,796,536
Final particle images (no.)	539,327	391,771	130,203	672,890	584,166
Map resolution (Å)	3.2	3.5	3.3	3.1	3.1
FSC threshold	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	3.0-4.2	3.3-4.4	3.0-4.8	2.9-4.1	2.9-4.2
Refinement					
Initial model used (PDB code)	3SN6	3SN6	3SN6	3SN6	3SN6
Model resolution (Å)	3.2	3.5	3.3	3.1	3.1
FSC threshold	0.143	0.143	0.143	0.143	0.143
Model resolution range (Å)	3.0-4.2	3.3-4.4	3.0-4.8	2.9-4.1	2.9-4.2
Map sharpening <i>B</i> factor (Å)	-109	-163	-106	-117	-106
Model composition					
Non-hydrogen atoms	8112	8112	8068	8128	8133
Protein residues	1035	1035	1035	1038	1038
Ligands	1	1	1	1	2
Lipid	1	1	0	1	1
<i>B</i> factor (Å)					
Protein	58.3	82.3	107	43.1	51.5
Ligand	68.1	81.4	115	46.4	55.1
Lipid	65.9	82.5	-	40.1	42.1
R.m.s. deviations					
Bond lengths (Å)	0.002	0.002	0.002	0.002	0.002
Bond angles (°)	0.51	0.52	0.54	0.47	0.51
Validation					
MolProbity score	1.97	2.05	1.83	2.17	1.97
Clashscore	13.4	12.4	10.4	13.4	20.22
Ramachandran plot					
Favored (%)	97.1	96.9	96.9	95.9	97.1
Allowed (%)	2.9	3.1	3.1	4.1	2.9

Table S2. Summary of ligand competition binding assays with DRD1. Related to the STAR Methods section “Radioligand binding assay”.

Mutation	Ki (nM)	Fold	n	Ki (nM)	Fold	n	Ki (nM)	Fold	n
	A77636			Fenoldopam			PW0464		
WT	1.55±0.34	1.0	4	5.47±0.63	1.0	4	14.34±0.418	1.0	4
D103 ^{3,32} A	ND	ND	3	ND	ND	3	ND	ND	3
S198 ^{5,42} A	ND	ND	3	ND	ND	3	ND	ND	3
S202 ^{5,46} A	3.58±0.33	3.2±0.22	3	26.43±1.16	4.83±0.21	3	33.26±2.61	2.32±0.18	3
K81 ^{2,61} A	1.87±0.22	1.21±0.14	3	8.66±1.19	1.58±0.22	3	931.89±76.12	64.98±5.31	3
W99 ^{3,28} F	1.83±0.06	1.19±0.04	3	10.31±1.592	1.88±0.29	3	475.52±60.02	33.16±4.19	3
W321 ^{7,43} A	ND	ND	3	ND	ND	3	ND	ND	3

ND means not detected. “—” means no measurement.

Table S3. Summary of A77636 and fenoldopam induced cAMP accumulations assays of DRD1. Related to the STAR Methods section “cAMP functional assays”.

Mutation	EC ₅₀ (nM)	Fold	E _{max} (%WT)	n	EC ₅₀ (nM)	Fold	E _{max} (%WT)	n	Expression level (%WT)
	A77636					Fenoldopam			
WT	7.67±1.13	1.00	100	6	0.44±0.04	1.00	100	6	100
D103 ^{3,32} A	663.80±246.60	119.30±44.31	12.79±1.21	3	ND	ND	ND	3	108.45 ± 9.13
S198 ^{5,42} A	452.60±202.40	81.34±36.38	90.71±0.58	3	20.65±1.06	46.84±2.40	62.48±2.39	3	112.23 ± 8.57
S202 ^{5,46} A	1203±298.40	216.20±53.62	95.43±3.54	3	1.24±0.29	2.80±0.67	69.87±4.048	3	93.56 ± 9.59
K81 ^{2,61} A	45.02±3.42	8.09±0.62	44.60±6.59	3	0.64±0.14	1.44±0.31	85.77±1.02	3	89.22± 7.63
W99 ^{3,28} F	54.03±5.73	9.71±1.03	95.08±2.57	3	1.41±0.13	3.21±0.29	73.78±4.78	3	118.37± 3.96
W321 ^{7,43} A	84.65±11.36	15.21±2.04	92.04±5.39	3	23.78±3.89	53.94±8.82	56.64±2.83	3	91.83± 8.46
N292 ^{6,55} H	732.10±364.40	131.50±65.48	47.26±10.11	3	11.55±0.88	26.19±2.00	40.27±2.92	3	102.75± 10.18
F313 ^{7,35} V	220.80±6.32	39.67±1.13	83.20±7.46	3	6.88±1.13	15.62±2.56	56.35±2.59	3	97.57± 6.28
F129 ^{1CL2} L	62.84±15.31	11.29±2.75	112.60±3.51	3	-	-	-		117.69± 9.13
F129 ^{1CL2} A	587.80±202.50	105.60±36.40	69.65±5.91	3	-	-	-		115.39 ± 5.32
A221 ^{5,65} L	301.60±21.99	54.20±3.95	95.88±8.98	3	7.52±0.34	15.35±1.84	97.33±3.65	3	97.23 ± 12.07
Q224 ^{5,68} R	280.80±34.48	50.45±6.19	96.28±5.56	3	1.41±0.12	2.88±0.42	96.80±7.62	3	74.89 ± 5.93
I225 ^{5,69} R	35.27±7.63	6.33±1.37	82.83±6.29	3	1.21±0.28	2.47±0.09	105.92±2.69	3	121.30 ±11.30
S199 ^{5,43} A	335.90±17.26	60.36±3.10	105.50±6.81	3	30.61±1.82	62.25±3.71	101.10±2.61	3	93.28 ± 9.27
S199 ^{5,43} C	556.80±57.54	100.00±10.34	99.71±1.78	3	691.40±66.46	>1000	100.60±3.52	3	83.46 ± 9.02
N292 ^{6,55} T	688.50±59.20	123.70±10.64	106.70±3.29	3	-	-	-		113.29 ± 8.69
N292 ^{6,55} L	524.00±58.44	94.15±10.50	105.70±3.56	3	-	-	-		95.11± 9.13

ND means not detected. “-” means no measurement.

Table S4. Summary of PW0464 induced cAMP accumulations assays of DRD1. Related to Figure 3C.

Mutation	EC₅₀ (nM)	Fold	E_{max} (%WT)	n	Expression level (%WT)
WT	1.81±0.34	1.00	100	6	100
D103^{3,32}A	659.10±44.37	519.00±34.94	89.12±3.77	3	108.45 ± 9.13
D103^{3,32}N	15.01±1.70	11.82±1.34	100.09±1.60	3	98±3.45
D103^{3,32}L	ND	ND	ND	3	101±9.33
S198^{5,42}A	19.34±3.12	15.23±2.53	99.91±5.52	3	112.23 ± 8.57
S202^{5,46}A	11.89±0.91	9.36±0.71	106.50±12.44	3	93.56 ± 9.59
K81^{2,61}A	42.04±15.21	33.10±11.97	72.00±8.89	3	89.22± 7.63
W99^{3,28}F	22.75±0.58	17.91±0.45	82.88±10.37	3	118.37± 3.96
W321^{7,43}A	65.67±5.48	51.74±4.31	80.58±2.07	3	91.83± 8.46
N292^{6,55}H	244.70±46.76	192.70±36.81	95.23±2.51	3	102.75± 10.18
F313^{7,35}V	21.39±0.16	16.84±0.13	98.84±4.92	3	97.57± 6.28
A221^{5,65}L	78.68±5.23	61.95±4.12	58.78±5.76	3	97.23 ± 12.07
Q224^{5,68}R	39.47±4.56	31.08±3.59	73.80±4.11	3	74.89 ± 5.93
I225^{5,69}R	49.88±6.30	39.28±4.96	72.72±2.65	3	121.30 ± 11.30

ND means not detected. “–” means no measurement.

Table S5. Summary of dopamine and SKF83959 induced cAMP accumulations assay of DRD1. Related to the STAR Methods section “cAMP functional assays”.

Mutation	EC ₅₀ (nM)	Fold	E _{max} (%WT)	n	EC ₅₀ (nM)	Fold	E _{max} (%WT)	n	
	Dopamine					SKF83959			
WT	12.51±1.38	1.00	100	6	0.30±0.02	1.00	100	6	
D103^{3,32}A	ND	ND	ND	3	ND	ND	ND	3	
S198^{5,42}A	1521±228.70	156.10±23.47	87.66±10.07	3	487.10±45.22	1750±163.80	85.01±2.01	3	
S202^{5,46}A	2645±356.50	271.40±36.58	104.10±11.54	3	70.61±11.07	255.80±40.12	82.33±0.36	3	
S199^{5,43}A	976.70±201.20	96.22±3.55	96.02±3.17	3	31.63±0.94	114.60±3.42	100.01±4.33	3	
S199^{5,43}C	ND	ND	ND	3	1226±30.87	>2000	97.78±4.62	3	
N292^{6,55}H	ND	ND	ND	3	-	-	-	3	
S107^{3,36}A	2372±297.70	243.40±30.55	59.02±2.24	3	-	-	-	3	

ND means not detected. “-” means no measurement.