

Supplementary Online Materials

for

Fusion Partner Toolchest for the Stabilization and Crystallization of G Protein-Coupled Receptors

Eugene Chun^{1#}, Aaron A. Thompson^{1#}, Wei Liu^{1#}, Christopher B. Roth², Mark T. Griffith²,
Vsevolod Katritch¹, Joshua Kunken¹, Fei Xu¹, Vadim Cherezov¹, Michael A. Hanson^{2*},
Raymond C. Stevens^{1*}

¹Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines
Road, La Jolla, CA 92037, USA

²Receptos, 10835 Road to the Cure, Suite #205, San Diego, CA 92121, USA

These authors contributed equally to this work

*Address for correspondence: mhanson@receptos.com; stevens@scripps.edu

Inventory

Table S1 is an example table of PDB screen results from which the domains shown in figure 1 were selected.

Figure S1 shows that the multiple receptor bands can be consolidated with the addition of PNGaseF and reducing agent using A_{2A}AR-BRIL (construct 16) and β₂AR-BRIL (construct 24) as representative examples.

Figure S2 shows complementary FRAP data that was used to guide the crystallization trials in figure 6.

Table S1. PDB search results for potential GPCR fusion partners, related to figure 1. PDB

entry results as determined by the search criteria in the main text. PDB entries are sorted by resolution.

No	PDB ID	PDB HEAD	Res	Bfactor	Heteroatoms	Swiss ID	Charge	Mass	Dist (NC)
1	2dsx	ELECTRON TRANSPORT	0.68	7.5	FE	RUBR_DESGI	-6.	5342	11.2
2	1w0n	HYDROLASE	0.8	8.6	CA,MG,SO4	XYND_PAEPO	-5.	12470	7.8
3	2o7a	HYDROLASE	0.84	6.2	ACT,CL,SME	LYS_BPT4	4.	15914	10.1
4	1rb9	IRON-SULFUR PROTEIN	0.92	9.2	FE2,FME,SO4	RUBR_DESVH	-6.	6354	10.7
5	2fdn	ELECTRON TRANSPORT	0.94	8.5	SF4	FER_CLOAC	-8.	6450	5.8
6	2xom	HYDROLASE	0.95	7.8			-3.	16791	9.1
7	2xod	FLAVOPROTEIN	0.96	12.9			-4.	13415	10.1
8	3fsa	METAL BINDING PROTEIN	0.98	7.8	CU1	AZUR_PSEAE	-3.	13109	10.7
9	2zpm	Hydrolase	0.98	10.9	MLY,MSE	RSEP_ECOLI	-5.	9688	12.1
10	2gkg	SIGNALING PROTEIN	1.	10.6			-3.	14981	9.1
11	2qsk	SUGAR BINDING PROTEIN	1.	11.3	CL,GOL	SVN_SCYVA	4.	9861	5.6
12	1c7k	HYDROLASE	1.	12.	CA,ZN	SNPA_STRCS	-5.	13450	7.6
13	1nkd	TRANSCRIPTION REGULATION	1.09	16.4		ROP_ECOLX	-4.	7253	6.2
14	2nwf	OXIDOREDUCTASE	1.1	7.9	FES,GOL	UCRI_RHOSH	-5.	15970	9
15	2aqm	OXIDOREDUCTASE	1.1	9.6	CU1,GOL,SO4,ZN	SODC_BRUAB	-5.	15422	12.8
16	2rhf	HYDROLASE	1.1	13.2	MSE,PO4		-4.	7878	10
17	2ccw	ELECTRON TRANSPORT (CUPROPROTEIN)	1.13	17.1	CU1	AZUR2_ALCX X	0.	13759	9.5
18	3tbn	METAL BINDING PROTEIN	1.15	12.1			4.	9187	10.9
19	2xfd	SUGAR BINDING PROTEIN	1.19	7.6			-3.	10961	7.7
20	2h8e	HYDROLASE	1.2	12.9		RUSA_ECOLI	6.	12850	12.3
21	1usm	TRANSCRIPTIONAL STIMULATOR	1.2	15.1			0.	8698	13.3
22	2yh5	LIPID BINDING PROTEIN	1.25	16.8			-5.	12558	11.8
23	1jni	OXIDOREDUCTASE	1.25	22.8	HEM	NAPB_HAEIN	4.	6338	12.5
24	1rtt	Structural genomics, unknown function	1.28	11.6	SO4		-2.	17487	10
25	1zce	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.3	9.3	MSE		-4.	15706	4.7
26	3nbm	TRANSFERASE	1.3	10.5			0.	11518	14
27	2ehs	LIPID TRANSPORT	1.3	13.9	NA,ZN	ACP_AQUAE	-14.	7885	12.1
28	1vmh	UNKNOWN FUNCTION	1.31	17.	NA		-4.	13332	9.8
29	1oh4	HYDROLASE	1.35	12.5	BMA,CA,GLA,GOL,SO4		-6.	18776	7.6
30	2ip6	ANTIMICROBIAL PROTEIN	1.35	13.6	SO4		-1.	9202	12.9
31	2wds	TRANSFERASE	1.35	14.8		ACPS_STRCO	-2.	12128	4.6
32	1wck	STRUCTURAL PROTEIN	1.36	14.7	CAC		-5.	12798	14.9
33	2nuh	UNKNOWN FUNCTION	1.39	16.4	IMD		-3.	10811	14.8
34	2fi1	HYDROLASE	1.4	12.3	CA		-14.	19652	8.3
35	3bzq	SIGNALING PROTEIN/ TRANSCRIPTION	1.4	13.9		GLNB_MYCTU	-1.	9782	13.3
36	2z98	Oxidoreductase	1.4	17.	EDO,FMN	AZOR_ECOLI	-6.	19394	14.5
37	2y39	METAL BINDING PROTEIN	1.41	13.5			-4.	11731	6.2
38	1mb3	SIGNALING PROTEIN, CELL CYCLE	1.41	15.3	MG		-9.	12344	14.9
39	3by8	TRANSFERASE	1.45	22.3	MLT	DCUS_ECOLI	0.	15150	12.4

No	PDB ID	PDB HEAD	Res	Bfactor	Heteroatoms	Swiss ID	Charge	Mass	Dist (NC)
40	3i7m	HYDROLASE	1.46	14.1	MSE		-10.	15812	11.4
41	3a0s	Transferase	1.47	24.8	PEG,PG4,PGE		-1.	10791	5.5
42	2j1a	HYDROLASE	1.49	20.7	CA,GAL	OGA_CLOPE	-7.	14467	7.6
43	3kyz	TRANSFERASE	1.49 7	19.3	CL,FMT,MSE	PFES_PSEAE	-4.	12734	4.8
44	3akb	METAL BINDING PROTEIN	1.5	13.5			-8.	16492	9.5
45	2vmh	SUGAR-BINDING PROTEIN	1.5	15.2	CA		-13.	14767	11.3
46	2wz8	SUGAR BINDING PROTEIN	1.5	15.5			-1.	14055	6.8
47	3po8	PEPTIDE BINDING PROTEIN	1.5	15.9			-3.	10008	7.4
48	2g2c	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.5	16.	MSE,NA		-4.	15764	13.6
49	1f7l	TRANSFERASE	1.5	16.3	CA,CL,COA	ACPS_BACSU	6.	12138	5.2
50	2y7l	LYASE	1.5	16.5			-7.	14315	10.9
51	2qvg	TRANSFERASE	1.5	16.8	MSE		-4.	13859	13.5
52	1fhm	ELECTRON TRANSPORT	1.5	17.2	FE2	RUBR_CLOPA	-8.	5520	11.6
53	2qsw	HYDROLASE	1.5	17.8	GOL,MSE,ZN	METN2_ENTFA	-5.	10556	11.9
54	1lo7	HYDROLASE	1.5	18.7	4CO,EDO	4HBT_PSEUC	0.	15082	7.4
55	1ln4	RNA BINDING PROTEIN	1.5	20.6		YHBY_ECOLI	1.	10112	14.2
56	2hx5	HYDROLASE	1.5	21.	ETX,MSE	DNCH_PROMM	-5.	16323	13.1
57	2w1r	TRANSCRIPTION	1.5	21.7		SP5T_BACSU	-10.	11982	14.7
58	1oal	OXIDOREDUCTASE	1.5	23.7	CU,ZN	SODC_PHOLE	-4.	14693	12.2
59	1y9l	LIPID BINDING PROTEIN	1.5	24.2	ACT,UND	MXIM_SHIFL	3.	12084	12.9
60	1x3o	LIPID TRANSPORT	1.5	24.2		ACP_THET8	-12.	8505	7.7
61	3dau	OXIDOREDUCTASE	1.5	25.	MTX,NAP	DYR_ECOLI	-10.	16515	13.8
62	1vmj	TRANSFERASE	1.52	16.8	NA,SO4		-3.	15420	5.6
63	2hx0	DNA BINDING PROTEIN	1.55	16.4	MG,MSE		-4.	14002	10
64	3osx	CHAPERONE	1.55	19.9			-6.	19132	13
65	2c4w	LYASE	1.55	26.	GAJ,GOL,IMD	AROQ_HELPY	-6.	18684	6.6
66	5nul	ELECTRON TRANSPORT	1.6	11.6	FMN	FLAV_CLOBE	-16.	14908	11.5
67	2asf	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.6	11.8	CIT,MSE,NA	Y2074_MYCTU	0.	12759	5.3
68	1u84	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.6	13.7	EDO,GOL		-4.	8483	11.9
69	3gwi	Hydrolase	1.6	14.8	SO4	ATMA_ECOLI	-6.	17355	5.6
70	1a3c	TRANSCRIPTION REGULATION	1.6	16.7	SM,SO4	PYRR_BACSU	-4.	18168	9.7
71	2o1a	SURFACE ACTIVE PROTEIN	1.6	18.8	EDO,MSE,SO4	ISDA_STAAM	3.	14303	10.2
72	1cuo	ELECTRON TRANSPORT	1.6	19.2	CU	AZUR2_METJ	-1.	12862	11.5
73	2w3w	OXIDOREDUCTASE	1.6	20.6	NDP,VG9		-2.	17283	13.8
74	2x5p	PROTEIN BINDING	1.6	21.8			-5.	10379	8
75	1id0	TRANSFERASE	1.6	23.2	ANP,MG	PHOQ_ECOLI	-8.	16220	8.9
76	2in0	HYDROLASE	1.6	23.3		RECA_MYCTU	-2.	14295	7.9
77	1gvp	DNA-BINDING PROTEIN	1.6	30.9		G5P_BPF1	3.	9140	11.8
78	1pzs	oxidoreductase, metal binding protein	1.63	15.	CU	SODC_MYCTU	-4.	16154	14.5
79	2i6v	PROTEIN TRANSPORT, MEMBRANE PROTEIN	1.63	16.		GSPC_VIBCH	-7.	9392	7
80	1gny	CARBOHYDRATE-BINDING MODULE	1.63	17.7	NA,XYP		-14.	14871	6.2
81	3i4q	HYDROLASE	1.63	19.1	MSE,NA		-12.	18710	13.6
82	1h98	ELECTRON TRANSPORT	1.64	18.	F3S,SF4	FER_THET8	-9.	7981	13.6
83	1j7g	HYDROLASE	1.64	23.3		DTD_HAEIN	-1.	14709	5.4
84	3bn7	UNKNOWN FUNCTION	1.64	25.9	ACT,MSE		0.	11532	5.8
85	1cxy	ELECTRON TRANSPORT	1.65	19.5	HEM	CYB5_ECTVA	-5.	8484	13.1
86	2a9o	SIGNALING PROTEIN	1.65	23.2	BEF,MN		-5.	12476	13.4

No	PDB ID	PDB HEAD	Res	Bfactor	Heteroatoms	Swiss ID	Charge	Mass	Dist (NC)
87	1wub	LIPID BINDING PROTEIN	1.65	23.3	OTP		-5.	17889	8.3
88	3bs3	DNA BINDING PROTEIN	1.65	29.2	EDO,MSE,SO4		4.	7935	8.6
89	1qjp	OUTER MEMBRANE	1.65	39.2	C8E	OMPA_ECOLI	-1.	14473	10.7
90	3s6m	ISOMERASE	1.65 1	15.			-5.	16596	12.1
91	3a0u	SIGNALING PROTEIN	1.66	14.	BEF,MG,PG4		-7.	12730	10.8
92	3kp8	OXIDOREDUCTASE	1.66	18.8			-3.	9326	13.6
93	3t1s	SIGNALING PROTEIN	1.67	23.3			-5.	14503	4.4
94	3h1g	SIGNALING PROTEIN	1.7	7.7		CHEY_HELPY	-2.	12736	7.9
95	1j27	structural genomics, unknown function	1.7	11.6			1.	10753	5.8
96	1ctf	RIBOSOMAL PROTEIN	1.7	14.3	SO4	RL7_ECOLI	-2.	6408	8.8
97	1fxd	ELECTRON TRANSFER(IRON-SULFUR)	1.7	16.	F3S,SCH	FER_DESGI	-15.	5894	5.2
98	1czn	ELECTRON TRANSPORT	1.7	17.2	FMN	FLAV_SYNE7	-17.	17387	11.8
99	1h75	ELECTRON TRANSPORT	1.7	20.6		NRDH_ECOLI	1.	8241	13.2
100	1df7	OXIDOREDUCTASE	1.7	21.4	GOL,MTX,NDP,S O4	DYR_MYCTU	-1.	16407	9.9
101	3gw m	TRANSFERASE	1.7	22.2		ACPS_MYCS2	-4.	14114	5.8
102	1wka	LIGASE	1.7	24.4		SYV_THETH	-6.	15011	5.5
103	1nza	structural genomics, unknown function	1.7	25.1	CL,GOL,NA,SO4	CUTA_THET8	-5.	10765	12.6
104	1tuv	UNKNOWN FUNCTION	1.7	26.	VK3	YGIN_ECOLI	-4.	10595	12.3
105	2g0c	HYDROLASE	1.7	29.1	SO4	DBPA_BACSU	3.	7297	7.5
106	2eg2	SIGNALING PROTEIN	1.72	20.9	ATP,CL	GLNB_AQUAE	-2.	9732	8.8
107	2huj	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.74	31.9	MSE		-3.	14052	13.8
108	3hgb	OXIDOREDUCTASE	1.75	10.1		GCSH_MYCTU	-16.	13372	12.2
109	1chd	CARBOXYL METHYLESTERASE	1.75	12.3		CHEB_SALTY	1.	19467	8.5
110	1o3u	UNKNOWN FUNCTION	1.75	17.4	MSE		-7.	12899	12.8
111	1uj8	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.75	17.5		ISCX_ECOLI	-12.	7949	14.7
112	1vjw	OXIDOREDUCTASE	1.75	18.7	SF4	FER_THEMA	-6.	5634	5.3
113	3bv8	TRANSFERASE	1.75	20.5	GOL,MSE,NA	DAPH_STAAM	-7.	10608	11.8
114	1ysq	GENE REGULATION	1.75	25.9	PO4	YIAJ_ECOLI	-6.	18710	10.6
115	1vr8	SIGNALING PROTEIN	1.75	26.7	AZI,GOL,MSE		3.	14652	8.4
116	3klb	FLAVOPROTEIN	1.75	27.3			-2.	17993	5.3
117	3qy3	HYDROLASE	1.75	32.2			-6.	14050	10.4
118	1nps	SIGNALING PROTEIN	1.8	9.3	CA	DESS_MYXXA	-4.	8701	11.4
119	1lop	COMPLEX (ISOMERASE/PEPTIDE)	1.8	13.9	NIT,SIN	PIIB_ECOLI	-6.	17226	5.4
120	1bdo	TRANSFERASE	1.8	14.	BTN	BCCP_ECOLI	-5.	8177	13.2
121	1jwq	HYDROLASE	1.8	15.2	ZN		1.	17983	13.1
122	2o0q	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.8	18.2			-8.	11495	12.3
123	2pln	SIGNALING PROTEIN	1.8	20.2	MSE		-4.	12423	13
124	3ls0	PHOTOSYNTHESIS	1.8	20.6			-1.	11916	9.5
125	1bxv	COPPER PROTEIN	1.8	20.9	CU	PLAS_SYNE7	-6.	9178	13
126	2cvb	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.8	21.7	MSE		-8.	19832	3.9
127	2qr3	TRANSCRIPTION	1.8	23.9			-4.	12606	13.4
128	1v2z	circadian clock protein	1.8	24.		KAIA_THEVL	-2.	11654	7.1
129	1zuh	Transferase	1.8	25.7		AROK_HELPY	-2.	15792	13
130	3pzy	BIOSYNTHETIC PROTEIN	1.8	26.6			-4.	13835	13.5

No	PDB ID	PDB HEAD	Res	Bfactor	Heteroatoms	Swiss ID	Charge	Mass	Dist (NC)
131	2qni	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.8	27.4	MSE		-6.	19826	13.2
132	2j22	CARBOHYDRATE-BINDING PROTEIN	1.8	28.6	CA,GOL		-7.	14447	5.5
133	2b8i	LIPID BINDING PROTEIN	1.8	30.			-5.	7951	9.4
134	1s7i	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.8	33.1			-4.	12660	9.8
135	3lqn	Structural Genomics, Unknown function	1.8	36.5			-5.	16141	10.1
136	1ku3	TRANSCRIPTION	1.8	38.2			3.	6368	14.4
137	3qra	CELL INVASION	1.80 1	33.6			1.	15458	9.4
138	3q1h	OXIDOREDUCTASE	1.80 4	20.2			-10.	17453	14.9
139	2ewt	DNA BINDING PROTEIN	1.81	13.9	SO4		2.	7255	11.8
140	3bci	oxidoreductase	1.81	33.1			3.	18346	13.5
141	1m6t	ELECTRON TRANSPORT	1.81	36.6	SO4	C562_ECOLX	-4.	10929	13.7
142	2cgg	PROTEIN TRANSPORT	1.83	29.5			-7.	7969	11.7
143	2xht	SUGAR BINDING PROTEIN	1.83 2	29.5			-1.	15986	9.6
144	3fxh	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.83 7	38.5	MSE		-4.	12060	13.7
145	3nph	PHOTOSYNTHESIS	1.84 9	24.7			-7.	14341	13.6
146	3sny	METAL BINDING PROTEIN	1.85	14.6			-2.	9094	12.6
147	2aan	ELECTRON TRANSPORT	1.85	18.8	CU,SO4	AURA_CHLAA	-4.	11877	14
148	2fwt	ELECTRON TRANSPORT	1.85	22.6	HEM		-3.	10961	9
149	2gw8	SIGNALING PROTEIN	1.85	27.			-2.	9960	12.7
150	2o4d	UNKNOWN FUNCTION	1.85	28.8	MSE		-4.	15273	7.3
151	2qpz	METAL BINDING PROTEIN	1.85	31.7	FES	NDOA_PSEPU	-8.	10859	5.6
152	3enu	STRUCTURAL PROTEIN	1.86	9.8			-1.	12034	13.3
153	1o50	TRANSFERASE	1.87	38.4			-6.	14994	8.4
154	1u2p	HYDROLASE	1.9	13.6	CL	PTPA_MYCTU	-4.	16006	11.5
155	2ywd	TRANSFERASE	1.9	18.		PDXT_THET8	-5.	19400	10.1
156	3dml	OXIDOREDUCTASE	1.9	18.2	MSE		-6.	10863	10.7
157	2pii	SIGNAL TRANSDUCTION PROTEIN	1.9	18.8		GLNB_ECOLI	-3.	11509	5.2
158	2cdn	TRANSFERASE	1.9	20.8	ADP,MG	KAD_MYCTU	-5.	18936	8.3
159	1xhd	TRANSFERASE	1.9	20.9	MSE,SO4		-2.	18364	5.9
160	2p1l	TRANSCRIPTIONAL REGULATOR	1.9	23.4	BEF,MG,MSE,PT	PHOP_ECOLI	-10.	13002	13.2
161	1r1m	MEMBRANE PROTEIN	1.9	24.5	TRS	OMP4_NEIMA	4.	14235	7.5
162	1q8b	Structural Genomics, Unknown Function	1.9	24.7	MSE	YJCS_BACSU	-6.	10562	3.8
163	1gui	CARBOHYDRATE BINDING MODULE	1.9	25.1	BGC,CA,GOL		-13.	16474	13
164	1tmy	CHEMOTAXIS	1.9	32.		CHEY_THEMA	0.	11980	7
165	3onp	TRANSFERASE	1.9	38.6			1.	18114	13.9
166	1tuw	UNKNOWN FUNCTION	1.9	38.7	SO4	TCML_STRGA	-9.	11205	14.2
167	3le0	BLOOD CLOTTING	1.91	24.5			-3.	14647	7.9
168	3i7t	UNKNOWN FUNCTION	1.93	9.3			-2.	11567	13.9
169	1opc	TRANSCRIPTION REGULATION	1.95	22.9		OMPR_ECOLI	1.	10629	13.2
170	3hqj	TRANSFERASE	1.95	24.1	COA,MG	ACPS_MYCTU	-3.	11319	6.4
171	1jrl	HYDROLASE	1.95	29.	IMD,SO4	TESA_ECOLI	-1.	18556	12.1
172	2bbe	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	1.97	13.6	SO4		-5.	11900	6.2

No	PDB ID	PDB HEAD	Res	Bfactor	Heteroatoms	Swiss ID	Charge	Mass	Dist (NC)
173	2z2i	HYDROLASE	1.98	31.7		PTH_MYCTU	6.	17601	11.1
174	1f3z	PHOSPHOTRANSFERASE	1.98	37.8	ZN	PTGA_ECOLI	-10.	14559	6.8
175	1dur	ELECTRON TRANSPORT	2.	12.2	SF4	FER_PEPAS	-7.	5180	9.1
176	1i1o	ELECTRON TRANSPORT	2.	13.3	FMN	FLAV_DESVH	-19.	14895	11
177	2b45	HYDROLASE	2.	15.	EPE	XYNA_BACSU	2.	19060	7.2
178	3gce	OXIDOREDUCTASE	2.	17.8	FES		-9.	10309	12.2
179	3f2v	structural genomics, unknown function	2.	18.	FMN		0.	18320	9.6
180	1tig	RIBOSOME BINDING FACTOR	2.	18.9		IF3_BACST	5.	9428	9.4
181	1spv	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	2.	20.9	MES,MSE	YMDB_ECOLI	-5.	17056	12.9
182	3dcm	TRANSFERASE	2.	23.3	SAM	Y1570_THEMA	-3.	19903	10.2
183	2fw5	ELECTRON TRANSPORT	2.	24.7	HEM		-2.	12198	10.4
184	1o22	UNKNOWN FUNCTION	2.	26.1	MSE		-7.	16453	5.7
185	1eso	OXIDOREDUCTASE	2.	28.	CU,ZN	SODC_ECOLI	-6.	14639	13.3
186	1mjc	TRANSCRIPTION REGULATION	2.	29.5		CSPA_ECOLI	-1.	6772	14.3
187	2hp7	Signaling Protein	2.	29.8			-10.	19165	14.5
188	3hz7	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	2.	31.9	MSE,SX		-5.	7423	7.7
189	1mi8	Hydrolase	2.	32.1		DNAB_SYNY3	0.	14553	4.4
190	2vjw	HYDROLASE	2.	32.5	MSE		-8.	13300	12.1
191	1vku	BIOSYNTHETIC PROTEIN	2.	33.4	MSE		-10.	9275	13.6
192	2es9	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	2.	36.2	MSE		-4.	10401	8
193	3cp3	STRUCTURAL GENOMICS, UNKNOWN FUNCTION	2.	37.9	ACY		-4.	14553	8.1

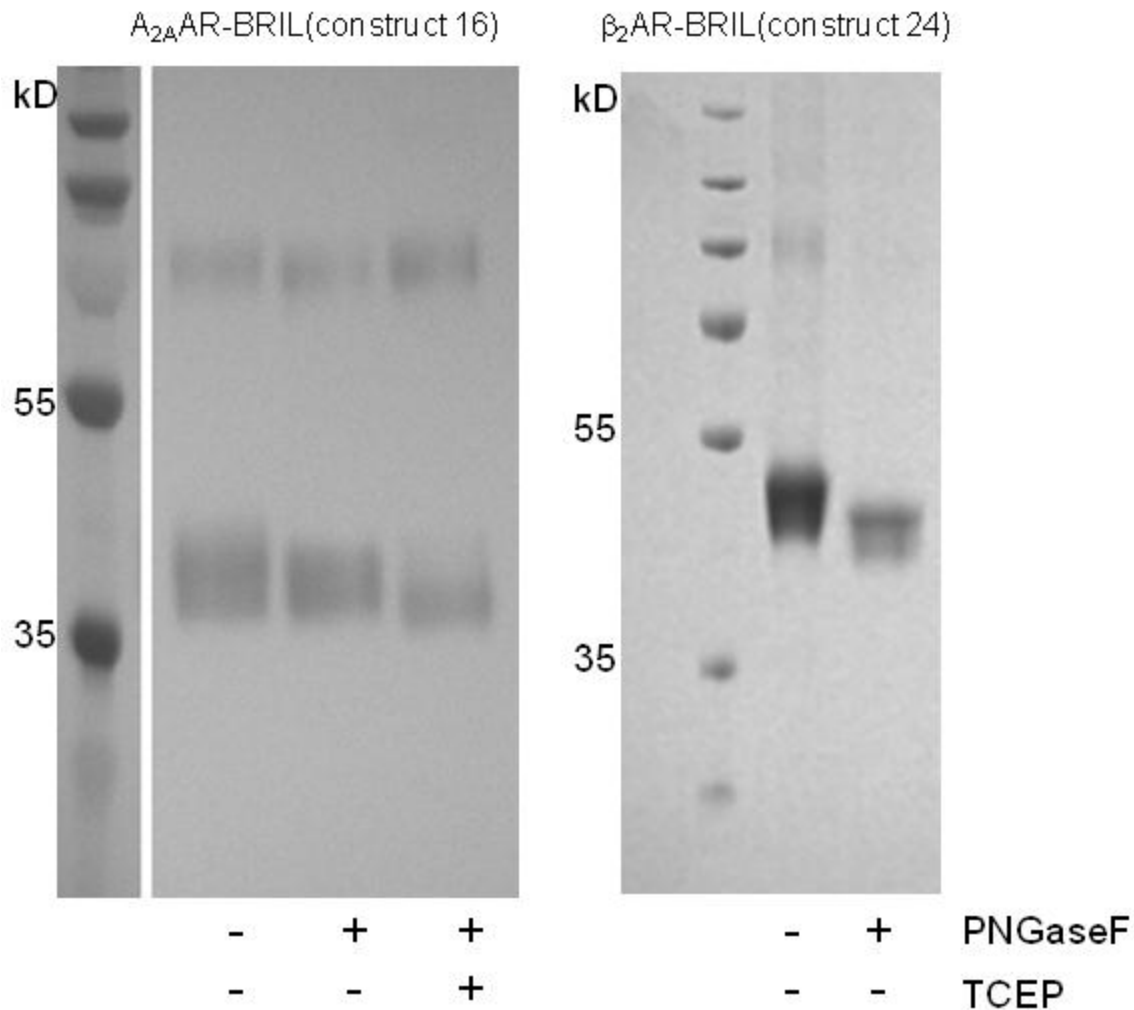


Figure S1: Deglycosylation experiments to test that multiple gel bands are due to different glycosylation states of the receptor, related to figure 3. α -FLAG western is shown. Using $A_{2A}AR$ -BRIL (construct 16) and β_2AR -BRIL (construct 24) as representative examples, multiple gel bands can be consolidated by the incubation of protein with PNGaseF overnight, and in the case of $A_{2A}AR$, with the further addition of tris(2-carboxyethyl)phosphine (TCEP) upon gel loading.

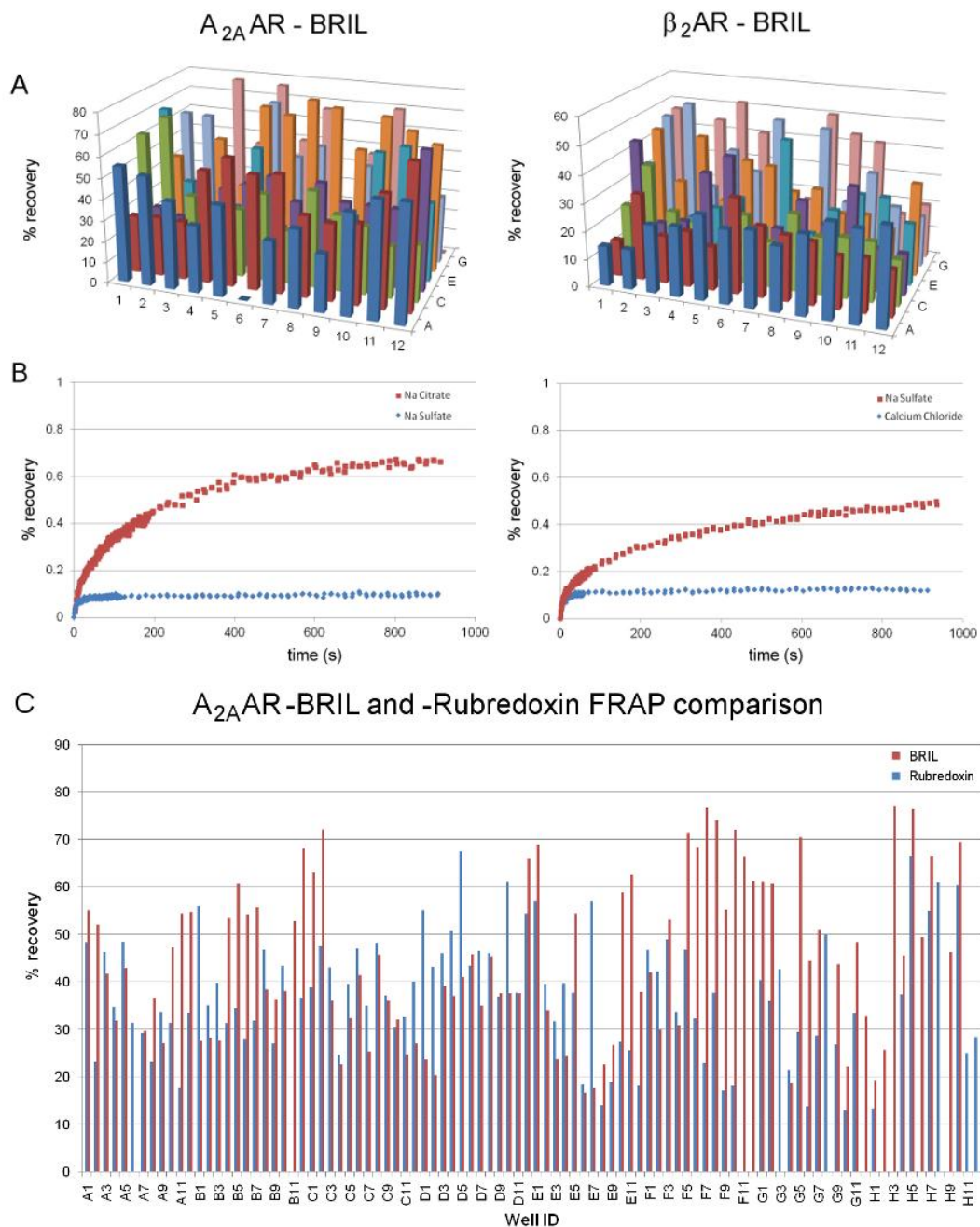


Figure S2: FRAP experiments were carried out for $A_{2A}AR$ -BRIL and β_2AR -BRIL to assay for diffusion, related to figure 7. (A) High throughput FRAP analysis showing good diffusion recovery in homemade salt screens at pH 5.0 and pH 7.0 for $A_{2A}AR$ -BRIL and β_2AR -BRIL, respectively. (B) Representative full FRAP experiments showing conditions providing high and low diffusion recovery for $A_{2A}AR$ -BRIL and β_2AR -BRIL for comparison. (C) FRAP comparison of $A_{2A}AR$ -rubredoxin (construct 7) and $A_{2A}AR$ -BRIL (construct 16). Over several conditions, $A_{2A}AR$ -BRIL in general showed higher diffusion recovery than $A_{2A}AR$ -rubredoxin.