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Supplementary Data Table 1a.

Statistics of XFEL data collection and processing					
Resolution (Å)	Completeness (%)	Multiplicity	SNR	CC*[a]	R_{split} (%)[b]
31.5-7.09	99.98	835.2	14.13	0.997	6.98
7.09-5.63	100	536.8	6.76	0.992	14.58
5.63-4.93	100	421.7	4.36	0.991	20.59
4.93-4.48	100	400.0	3.70	0.990	24.88
4.48-4.16	100	275.5	2.54	0.982	37.47
4.16-3.91	100	174.3	1.53	0.956	69.85
3.91-3.72	87.47	126.4	0.99	0.896	114.96
3.72-3.55*	42.32	133.0	1.25	0.884	91.75
3.55-3.42*	20.84	142.8	1.44	0.856	74.39
3.42-3.30*	6.40	116.4	1.73	0.874	58.77
Overall	76.30	383.3	4.70	0.996	19.10
Statistics of synchrotron data collection and processing					
Resolution (Å)	Completeness (%)	Multiplicity	I/σ	CC*[a]	R_{merge}(%)[c]
29.9-20.0	83.80	3.8253	11.49	1.000	4.90
20.0-15.2	100.00	4.4921	6.72	0.998	16.90
15.2-12.8	99.70	4.5217	5.22	0.998	23.40
12.8-11.2	99.50	4.9125	4.45	0.986	32.00
11.2-10.1	100.00	5.0651	4.52	0.985	32.10
10.1-9.3	100.00	5.2020	3.27	0.977	46.50
9.3-8.6	99.80	4.6953	2.51	0.968	55.00
8.6-8.1	100.00	4.9146	1.57	0.862	91.20
8.1-7.7	90.80	4.1185	1.08	0.809	118.80
Overall	97.50	4.6807	3.79	1.0000	31.4

*The XFEL data was truncated anisotropically (3.8 Å in *a** and *b** directions, 3.3 Å along *c**), which resulted in better CC* and SNR, but lower completeness in high resolution shells.

[a] $CC^* = \sqrt{\frac{2CC_{1/2}}{1+CC_{1/2}}}$

[b] $R_{split} = \sqrt{2}\frac{\sum_{hkl}|I_{even}-I_{odd}|}{\sum_{hkl}|I_{even}+I_{odd}|}$

[c] $R_{merge} = \sum|I_{h,i} - I_h|/\sum I_{h,i}$

Supplementary Data Table 1b. Summary of data and refinement statistics

Data collection	XFEL
Wavelength (Å)	1.33
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	109.2, 109.2, 452.6
α, β, γ (°)	90, 90, 90
Resolution (Å)	31.5-3.3 (anisotropic truncation 3.8, 3.8, 3.3)
R_{split} **(%)**	19.1
SNR or I/σ	4.70
CC*	0.996
Completeness (%)	76.3
Multiplicity	383

Refinement	
Resolution range (Å)	31.0-3.3 (3.4-3.3)*
No. reflections (all/free set)	62,613/3,098
R_{work} / R_{free} **(%)**	25.2/29.3 (33.3/40.8)*
No. atoms	
Rhodopsin	10,344
Arrestin	10,840
T4L	3,481
B factors	
Wilson	112.0
Proteins	159.7
R.M.S. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.0
Ramachandran	
Favored (%)	96.3
Outliers (%)	0.0
Clash Score	1.47
MolProbity score	1.13

*Values in parentheses are for highest-resolution shell.

Supplementary Data Table 2. Structure solution and refinement in possible space groups

Space group	Cell (Å)	Nmol/asu	TFZ	R_{work}(%)	R_{free}(%)
$P4_3$	*a*=*b*=109.2, *c*=452.6	4	23.9	30.7	36.9
$P2_12_12_1$	*a*=*b*=109.2, *c*=452.6	4	51.7	25.1	31.6
$P2_1$	*a*=*b*=109.2, *c*=452.6	8	27.8	23.4	31.8
C2	*a*=*b*=109.2, *c*=452.6	4	10.0	44.6	47.8
C2	*a*=*b*=154.5, *c*=452.6	8	14.5	30.9	35.7

For each space group, the molecular replacement was performed using one Rho-Arr complex as the search template in Phaser (ref. 68), and refinements with twinning were carried out using REFMAC5 (ref. 70). Overall, the solution in $P2_12_12_1$ gave the best packing, TFZ-score, and R_{free} values.

Supplementary Data Table 3. Correlation of distance between the structure and the DEER measurements

The modeled distances between nitroxide spinlabels are based on the crystal structure of the rhodopsin-arrestin complex. R1 nitroxide sidechains were modeled into the structure using common R1 rotamers (ref. 89&90). The experimental DEER distances for arrestin are based on measurements of arrestin bound to phosphorylated, light activated rhodopsin (ref. 41). General agreement was observed between the models and the most probable distances observed in experimental DEER data.

Mutants	Modeled Distance (Å)	DEER Distance (Å) (most probable)	Nitroxide Rotamer Selection**
32R1/356R1	15.7	16.0	ttm/ttm
12R1/108R1	19.6	17.0	mpm/tmm
111R1/173R1	30.7	30.0	tpm/mtp
16R1/381R1	18.6	19.0	mtp/tmm
103R1/376R1	33.2	35.0	tpm/mmp
85R1/244R1	34.4	36.0	ttp/mtp
139R1/60R1	31.0	31.0	mmp/mtm
139R1/173R1	18.4	~17	mmp/mtp
139R1/197R1	54.1	53.5	mmp/ttp
139R1/227R1	48.7	48.5	mmp/mtm
139R1/244R1	35.6	35.0	mmp/mtp
139R1/251R1	33.7	33.5	mmp/tpm
139R1/267R1	48.2	48.0	mmp/tmp
157R1/173R1	34.4	34.5	tpp/mtp
173R1/240R1	32.7	33.0	mtp/ptm
197R1/267R1	28.7	27.0	ttp/tmp
244R1/272R1	37.1	37.0	mtp/tpm
139R1/344R1*	36.4	45.5	mmp/pmp
197R1/344R1	20.4	19.0	ttp/pmp
244R1/344R1	26.4	25.5	mtp/pmp
267R1/344R1	22.6	24.0	tmp/pmp
Rho 74R1/Arr 240R1	34.1	33.0	tpp/ptm
Rho 74R1/Arr 139R1	21.7	22.0	tpp/mmp
Rho 74R1/Arr 60R1	28.2	28.0	tpp/ptm

*Note: The DEER distance for 139R1 and 344R1 is not a mono distribution but has two peaks: one at 45.5 Å and the other at ~35 Å, closely matching the model distance of 36 Å.
** Nitroxide Rotamers are defined in references 89 and 90.

Supplementary Data Table 4. Interface residues between rhodopsin and arrestin

	residues in rhodopsin	residues in arrestin		residues in rhodopsin	residues in arrestin
ICL1	T70	I73	TM5	L226	L78
				T229	Y251
TM2	L72	I73		A233	Y251
				A233	R82
	R135	G77		Q236	T320
TM3	V139	Y251			
	C140	Y251			
			ICL3	Q237	R82
	K141	Y256		Q237	D83
	P142	V248		E239	Y126
	M143	R292		E239	R319
	M143	Y256			
	N145	S143	TM6	T242	F80
	N145	C144		A246	T79
ICL2	N145	G69		E249	L78
	N145	Y68			
	F146	L133	C-loop	V250	L78
	F146	K142			
	F146	C144	TM7	N310	M76
	R147	R292		N310	G77
				K311	M76
			H8	K311	V75
				Q312	I73
				Q312	M76

Residues that have intermolecular distances less than 4 Å in the crystal structure are shown.

Table 5 Summary of Crosslinking data